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Evaluation of CHROMagar *Candida* in the rapid identification of medically important species of *Candida*

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Abstract

Providing a quick and effective method of identification of the important human pathogen *Candida* species is a vital step in delivering prompt and efficient disease treatment and determining antifungal susceptibility profile. This study was conducted with the aim of evaluating the use of CHROMagar *Candida* in the rapid presumptive taxonomical identification of *C. albicans*, *C. parapsilosis*, and *C. krusei*. The previous taxonomic classification of 188 *Candida* species were reconfirmed through the standard traditional methods of yeast purification, morphological and colonial characterization, and standard biochemical tests of sugar assimilation and fermentation. The traditional methods reconfirmed that all isolates belonged to the genus *Candida*: *C. albicans* (126), *C. parapsilosis* (41), and *C. krusei* (21). CHROMagar *Candida* (CA), a selective medium claimed to differentiate *Candida* species based on colony color, was compared to the germ tube test (GTT) in the presumptive identification of *C. albicans*. The use of GTT only confirmed 100 out of the 126 (79%) *C. albicans* isolates as compared to the 116 (92%) *C. albicans* verified using the CA medium. The use of CA gave a sensitivity of 92.1%, specificity of 98.4%, and accuracy of 94.1% compared with the 79.4% sensitivity, 70.5% specificity, and accuracy of 86.2% obtained for GTT. Overall, the better method to use for rapid and presumptive identification of *C. albicans* and *C. parapsilosis* is CHROMagar *Candida*.

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Introduction

Candida species are typically part of the normal commensal flora in mammalian hosts (Chin *et al.*, 2016). However, its capacity to produce superficial or systemic infections depends on the host immune system and various risk factors (Kauffman *et al.*, 2011). The global incidence of *Candida* infection has been steadily increasing and members of the genus *Candida* are currently recognized as important human pathogens. Thus, the rapid and accurate identification of *Candida* species is essential in delivering timely and effective treatments (Cameron *et al.*, 2016), prediction of the species-specific primary resistance, and variable antifungal susceptibility (Page and Kurtzman, 2005; Posteraro *et al.*, 2011; Raju and Rajappa, 2011; Chin *et al.*, 2016).

Traditionally, the identification of members of the genus *Candida* within a clinical laboratory relied upon biochemical assays (Nemcova *et al.*, 2015) and the germ tube test (Hope and Frey, 1999). It commonly starts with the use of an isolation medium, Sabouraud dextrose agar (Odds, 1991). In order to differentiate *C. albicans* from other *Candida* species rapidly, the germ tube test is often employed (Deorukhkar *et al.*, 2012). When the yeast cannot be named with the previous methods, further tests such as culturing on Cornmeal Agar, carbohydrate assimilation, and fermentation tests are done (Warren and Hazen, 1999). These laborious methods are time consuming, expensive and not all laboratories have the appropriate facilities. Furthermore, these tests increase costs, user input, and the time to identify and perform directly from a culture plate.

Rapid identification of pathogenic yeasts and detection of polyfungal specimens is deemed vital especially in this day of growing invasive and often lethal candidal infections (Anaissie, 1992). A new differential culture medium called "CHROMagar *Candida*" was reported to allow the selective isolation of yeasts and the simultaneous identification of *C. albicans* and non-*C. albicans* species. It is a commercial culture medium which contains species-specific chromogenic substrates that allow individual colonies to be identified by color development and colony morphology during growth. The aim of the study is to evaluate the use of CHROM agar *Candida* in the rapid presumptive taxonomical identification of *C. albicans*, *C. parapsilosis*, and *C. krusei*.

Materials and methods

Reconfirmation and classification of Candida species using conventional methods and germ tube test

A total of 188 clinical *Candida* isolates which were previously collected and identified were subjected to species-level identification using traditional methods for confirmation. Characterization and identification of the isolates were done by observation of wet mounts, isolation and colony characterization on Sabouraud Dextrose Agar (SDA), determination of hyphal growth via the germ tube test, and biochemical characterization. The classical Wickerham and Burtom method of assimilation of the sugars cellobiose, lactose, maltose, raffinose, and sucrose were done as well as the fermentative utilization of maltose (to differentiate *C. albicans* from *C. parapsilosis*).

Use of CHROMagar Candida in rapid identification of Candida species

CHROM agar *Candida* (CA) was prepared according to the manufacturer's recommendation. To evaluate CA as a rapid presumptive *Candida* identification medium, three control species (*C. albicans, C. parapsilosis* and *C. krusei*) and the 188 *Candida* isolates were streaked onto quarter plates of CA media. Identification of the *Candida* cultures was done based on differential colony color, following the manufacturer's guidelines and the published data of Odds and Bernaerts (1994).

Comparison between germ tube test and CHROMagar Candida use in Candida species identification

The results obtained through CHROMagar *Candida* were compared with the results of the germ tube test as well as the biochemical characterization of the three *Candida* species. The parameters used in the comparison were sensitivity, specificity, and accuracy.

Results and discussion

Characterization and reconfirmation of taxonomical identification of the Candida isolates using the conventional methods

Cultural characterization of the *Candida* isolates 48th-hour growth on SDA (at 37 °C) showed that all colonies generally formed opaque, smooth, convex and white to yellow-colored colonies on SDA, similar to the general description for *Candida* species (Sumathi and Devipriya, 2016). Wet mount (morphological) observations of 24-hour old cultures of the isolates showed that all isolates exhibited circular to ovoid, budding cells which is generally characteristic of yeasts, including *Candid*a (Agu *et al.*, 2015; Aprianti *et al.*, 2015). As shown in Table 1, data from the germ tube test, a conventional rapid presumptive method for the identification of *C. albican*s, indicated that 53% (100/188) of the isolates were found positive for the formation of germ tubes in rabbit serum after two to four hours at 37 °C and were presumptively identified as *C. albicans* strains. Cultural and morphological characterizations, however, are not sufficient to differentiate the yeast isolates because of very similar colony and cell morphology.

Table 1. Germ tube test, sugar assimilation, and maltose fermentation characteristics of the *Candida* isolates.

Number of isolates	Maltose fermentation	Assimilation of sugars					Germ tube test positive	*Candida* species
		suc	raf	mal	lac	cel		
126	+	+	-	+	-	-	100	*C. albicans*
41	-	+	-	+	-	-	---	*C. parapsilosis*
21	-	-	-	-	-	-	---	*C. krusei*

Legend: cel – cellobiose; lac – lactose; mal – maltose; raf – raffinose; suc – sucrose.
+ - positive- - negative.

The conventional (or reference) method of identification of yeast isolates (presumed to be *Candid*a) up to the species level was done by employing, in addition to the cultural and morphological characterizations, biochemical characterization. This consisted of carbohydrate assimilation and maltose fermentation. On the basis of data obtained from the assimilation of five different carbon sources (cellobiose, lactose, maltose, raffinose, and sucrose), two major groups of previously identified *Candida* species were formed: *C. albicans/C. parapsilosis* (89% or 167/188 of the isolates*)* and *C. krusei* (11% or 21), Although the carbohydrate fermentation test is rarely performed because it often leads to false-positive reactions due to the presence of endogenous carbohydrates adsorbed in the cell wall (Koneman *et al.,* 1992), maltose fermentation was conducted because the carbohydrate assimilation tests failed to differentiate the *C. albicans* from the *C. parapsilosis* isolates. One hundred twenty-six of these isolates were maltose-fermenters and finally classified as *C. albicans*; the rest (41) which were non-maltose fermenters were considered *C. parapsilosis*. The rest of the 21 isolates were also subjected to the maltose fermentation and all were non-fermentative and were positively identified as C. *krusei*.

Rapid identification of Candida species using CHROMagar Candida

The differential colony appearance of *Candida* spp. on CA medium makes it extremely useful in their rapid presumptive identification. Table 2 shows the representative colony colors on CA of all 188 isolates previously identified by the reference method. Of the 188 isolates grown on CA at 37 °C for 48 hours, 62% (116/188) exhibited green colonies (ranging from mint-green to blue-green), which is characteristic of *C. albican*s. The medium was, thus, able to correctly identify 92.1% of all true *C. albicans* isolates, giving a sensitivity of 92.1% (116/126). The remaining ten true *C. albicans* isolates were observed to have different colony colors and were considered to be false negative results.

Table 2. Colony color distribution of the *Candida* isolates after incubation at 37°C on CHROMagar *Candida* for 48 hours.

Species of *Candida**			Colony color on CA
kru (21)	*para* (41)	*alb* (126)	
---	---	114	Mint-green
---	---	---	Yellow-green
---	---	---	Light-green
---	---	1	Moss-green
---	---	1	Blue-green
---	---	2	Lavender
21	---	---	Violet
---	---	4	Grayish-violet
---	---	2	Gray
---	1	1	Steel-blue
---	40	---	Cream
---	---	1	Peach-pink

*identified based on the reference method: carbohydrate assimilation and maltose fermentation.
Legend: *alb – C. albicans*; *para – C. parapsilosis*; *kru – C. krusei.*

Of the 41 isolates identified by the reference method as *C. parapsilosis,* 97.6% (or 40) exhibited cream-colored colonies on CA (the true-positives). Sensitivity, specificity and accuracy of CA for the presumptive identification of *C. parapsilosis* were, thus, 97.6%, 93.2% and 94.1%, respectively. Lavender to violet colonies were observed among 11% (21) of the isolates and were classified as *C. krusei*.

Three statistical parameters (sensitivity, specificity, and accuracy) were used to separately evaluate the germ tube test (specific for *C. albicans*) and CHROMagar *Candida,* for the rapid presumptive identification of *C. albicans* and other non-*C. albicans* species. Taxonomical identification of the isolates is based on the previous isolate identity and in conformity with the reference method employed in this study.

Germ tube test as rapid identification method for Candida albicans

The germ tube test (GTT) was only about 79.4% sensitive as it was able to identify only 100 of the 126 isolates proven to be *C. albicans*. GTT however, was found to be 70.5% specific because it identified 62 out of the 88 isolates proven to be non-*C. albicans*.

The overall accuracy of the test was 86.2%. GTT was a relatively faster method than the conventional test for the identification of *C. albicans* as the results were obtainable in just two to four hours. However, some limitations of the GTT as reported earlier (Rippon, 1974) were noted: (1) subjectivity and reliance to the expertise of the observer, (2) likely observation of false-positive results if cells were incubated for more than 4 hours (3) non-formation of germ tube by some of the *C. albicans* isolates, and (4) false-negative results due to a heavy inoculum.

Comparative analyses of the germ tube test and the use of CHRO Magar Candida for the rapid identification of Candida species

Since the GTT only confirmed 100 out of the 126 *C. albicans*, it has a sensitivity of 79.4%, which is relatively low compared to the *C. albicans* specificity of CA which is at 92.1% (116/126), making CA to be more preferable in rapid identification of *C. albicans*.

On the other hand, the specificity value for GTT is at 70.5% indicating that only 70.5% of the non-*C. albicans* strains have been correctly identified (isolates which did not exhibit germ tube formation). The corresponding specificity of CA was at 98.4%, meaning that this percentage of the true non-*C. albicans* isolates gave a non-green colony appearance on CA. The results yielded that CA is the preferential method in the identification of non-*C. albicans* over GTT.

The accuracy values of both GTT and CA were at 86.2% and 94.1%, respectively. These mean for GTT the true *C. albicans* and true non-*C. albicans* combined have been correctly determined at 86.2%. For CHROMagar *Candida*, the method was 94.1% accurate in identifying whether a strain was *C. albicans* or not.

In summary, the use of CA gave a sensitivity of 92.1%, specificity of 98.4%, and accuracy of 94.1% compared with the 79.4% sensitivity, 70.5% specificity, and accuracy of 86.2% obtained for GTT. The higher sensitivity noted for CA suggests that it would be a better method to use for the presumptive identification of *C. albicans*. For the detection of *C. parapsilosis*, CA gave a high sensitivity, specificity, and accuracy of 97.6%, 93.2% and 94.1%, respectively.

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