Identification of local clinical Candida isolates using CHROMagar Candida™ as a primary identification method for various Candida species

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Abstract. The objective of our study was to study the effectiveness of CHROMagar Candida™ as the primary identification method for various clinical Candida isolates, other than the three suggested species by the manufacturer. We studied 34 clinical isolates which were isolated from patients in a local teaching hospital and 7 ATCC strains. These strains were first cultured in Sabouraud dextrose broth (SDB) for 36 hours at 35ºC, then on CHROMagar plates at 30ºC, 35ºC and 37ºC. The sensitivity of this agar to identify Candida albicans, Candida dubliniensis, Candida tropicalis, Candida glabrata, Candida rugosa, Candida krusei and Candida parapsilosis ranged between 25 and 100% at 30ºC, 14% and 100% at 35ºC, 56% and 100% at 37ºC. The specificity of this agar was 100% at 30ºC, between 97% and 100% at 35ºC, 92% and 100% at 37ºC. The efficiency of this agar ranged between 88 and 100% at 30ºC, 83% and 100% at 35ºC, 88% and 100% at 37ºC. Each species also gave rise to a variety of colony colours ranging from pink to green to blue of different colony characteristics. Therefore, the chromogenic agar was found to be useful in our study for identifying clinical Candida isolates.

INTRODUCTION

Over the last decade, the common etiology of many fungal infections especially among immunocompromised patients was known to be Candida species. Diagnosis was simply based on whether it was Candida albicans or non-albicans Candida species which indicated the appropriate treatment for these fungal infections. On the other hand, it was reported that apart from C. albicans, other species that can cause infections in humans are Candida tropicalis, Candida parapsilosis, Candida krusei, Candida guilliermondii, Candida glabrata, Candida kefyr, Candida lusitaniae, Candida rugosa, Candida viswanathii and Candida stellatoidea (Basetti et al., 2007; Arora, 2004). They are present as normal microbiota in the human body, i.e. on skin, mouth, large intestines, urinary and reproductive systems (Tortora et al., 2007). They can cause diseases in humans when the physiological balance of the body is upset or the host's defence is in a compromised state. With the overuse of antibacterial agents, immunosuppressive agents, cytotoxins, irradiation and steroids, this new category of systemic mycoses has emerged. These patients have been deprived of immune resistance by the body's normal flora, thus develop opportunistic mycoses, for example candidiasis, aspergillosis, mucormycosis and others (Block & Beale, 2004). The conventional yeast identification method in a clinical laboratory is based on Sabouraud dextrose medium, which requires about 24 to 72 hours for the cultures to grow depending on the species. The identification of C. albicans is usually based on the formation of germ tube in human or animal serum at 37ºC for 3 hours (Schoofs et al., 1997). Following
plate cultures, Gram staining and carbohydrate assimilation tests were usually done to verify the species further which can be time consuming as they require at least 3 working days to obtain the results.

A new presumptive identification medium for *C. albicans*, *C. tropicalis* and *C. krusei* known as CHROMagar Candida™ was recently developed. This medium contains chromopeptone, glucose, chromogen mix, chloramphenicol and agar. There were many reports that evaluated the use of this medium for these *Candida* species (Beighton *et al.*, 1995; Bouchara *et al.*, 1996; Willinger & Manafi, 1998). We studied the effectiveness of CHROMagar Candida™ to identify various clinical *Candida* isolates obtained from a hospital in Kuala Lumpur, apart from the three species used for CHROMagar identification. Colony colours at various incubation temperatures and time periods were observed. We also investigated the variation of colours formed by several different *Candida* species than the three species recommended by the manufacturer. This primary identification of clinical *Candida* isolates could give an indication on the most appropriate antifungal drug for the treatment of candidiasis as some species are inherently resistant to some antifungal drugs. Colony characteristics of the strains on potato dextrose agar (PDA) were also observed and compared with the colonies formed on CHROMagar Candida.

**MATERIALS AND METHOD**

**Candida isolates**
The *Candida* isolates were obtained from patients with *Candida* infections from a local public hospital and were cultured on Sabouraud's dextrose agar (Difco, USA). A total of 34 clinical isolates were studied and 7 American Type Culture Collection (ATCC) strains were used in this study. All these strains were first subcultured in Sabouraud's dextrose agar (Difco, USA) at 35°C for 36 hours to check the viability of the fungi.

**Culture conditions**
The CHROMagar Candida™ was prepared according to the instructions given by the manufacturer (Becton Dickinson, USA). A loopful of an isolate was streaked on a CHROMagar plate and incubated at 35°C. The growth was checked every 24 hours until 72 hours of incubation and the colour of the colonies were noted by a single observer. The colony characteristics were also studied for each isolate.

**Incubation temperatures**
As a comparative study, the steps were repeated for incubation at different temperatures. One set of the strains was incubated at 30°C and another set was incubated at 37°C (following body temperature). All cultures were observed for their colony colours after 24 hours, 48 hours and 72 hours of incubation.

**Potato dextrose agar medium**
Potato dextrose agar (PDA) media were prepared according to the instruction by the manufacturer (Difco, USA) and poured into 9 cm Petri dishes. Clinical *Candida* isolates and reference strains were streaked on the agar individually and the plates were incubated at 35°C. Observation of the colony colour and characteristics was done 48 hours after incubation.

**Biochemical identification**
Biochemical identification of all the strains was performed with RapID™ Yeast Plus System biochemical test kits (Remel, USA). It was used as a gold standard for the identification of *Candida* species.

**Analysis**
To study the effectiveness of the CHROMagar Candida™ in species identification, the results of the CHROMagar were compared with the biochemical tests. The following analyses were used to obtain the sensitivity, specificity and efficiency of the CHROMagar Candida™.

Sensitivity = True positives/[True positives + False negatives] x 100%

Specificity = True negatives/[True negatives + False positives] x 100%

Efficiency = (True positives + True negatives)/ Total x 100%
RESULTS

The results are summarized in Table 1 and 2. Table 1 shows true positive results of the colony colours on CHROMagar Candida™ and Table 2 shows the characteristics of the colonies for each species. All the reference strains used from ATCC were identified with molecular identification at NCBI for confirmation. The colony characteristics and colour on CHROMagar were compared to these reference strains. The colony characteristics of strains belonging to the same species appeared to be the same from our observation. Colonies of *C. albicans* seemed to have an apple green colour, *Candida dubliniensis* had dark green appearance and *C. rugosa* were green. Although *C. krusei* and *C. glabrata* appeared to have the same shade (lavender or pink), but the colonies of *C. krusei* had velvety texture whereas colonies of *C. glabrata* seemed to have glossy texture. However, one *C. albicans* strain gave rise to greyish pink and one with cream coloured colonies, compared to the ATCC strain and other *C. albicans* strains which were apple green. Colonies of *C. parapsilosis* exhibited a variety of colours, from cream to pink to metallic blue to dark green. Isolates of *C. rugosa* appeared light green on CHROMagar at 30°C, dark green at 35°C and 37°C, but one isolate appeared as metallic blue at all three temperatures. The identity of all the isolates was confirmed with biochemical tests. We found that this metallic blue colony was *Candida tropicalis*.

Table 1. Effectiveness of CHROMagar Candida™ as a primary identification medium after 48 hours of incubation at 30°C, 35°C and 37°C, as compared with biochemical test kits (RapID™ Yeast Plus System, Remel USA)

<table>
<thead>
<tr>
<th>Species (n)</th>
<th>C. albicans (7)</th>
<th>C. dubliniensis (4)</th>
<th>C. tropicalis (5)</th>
<th>C. glabrata (3)</th>
<th>C. rugosa (9)</th>
<th>C. krusei (6)</th>
<th>C. parapsilosis (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (ºC)</td>
<td>30 35 37</td>
<td>30 35 37</td>
<td>30 35 37</td>
<td>30 35 37</td>
<td>30 35 37</td>
<td>30 35 37</td>
<td>30 35 37</td>
</tr>
<tr>
<td>True positive</td>
<td>5 5 5</td>
<td>1 4 4</td>
<td>5 5 5</td>
<td>2 3 3</td>
<td>7 9 5</td>
<td>6 6 6</td>
<td>2 1 5</td>
</tr>
<tr>
<td>True negative</td>
<td>34 34 34</td>
<td>37 37 34</td>
<td>36 36 36</td>
<td>38 38 37</td>
<td>32 32 33</td>
<td>35 35 33</td>
<td>34 34 32</td>
</tr>
<tr>
<td>False positive</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 0</td>
<td>0 0 1</td>
<td>0 0 1</td>
<td>0 0 0</td>
<td>0 1 2</td>
</tr>
<tr>
<td>False negative</td>
<td>2 2 2</td>
<td>3 0 0</td>
<td>0 0 0</td>
<td>1 0 0</td>
<td>2 0 4</td>
<td>0 0 0</td>
<td>5 6 2</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>71 71 71</td>
<td>25 100 100</td>
<td>100 100 100</td>
<td>67 100 100</td>
<td>78 100 56</td>
<td>100 100 100</td>
<td>29 14 71</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100 100 100</td>
<td>100 100 92</td>
<td>100 100 100</td>
<td>100 100 97</td>
<td>100 100 97</td>
<td>100 100 94</td>
<td>100 97 94</td>
</tr>
<tr>
<td>Efficiency (%)</td>
<td>95 95 95</td>
<td>93 100 93</td>
<td>100 100 100</td>
<td>98 100 98</td>
<td>95 100 88</td>
<td>100 100 100</td>
<td>88 83 90</td>
</tr>
</tbody>
</table>

Table 2. Colony characteristics of various *Candida* species on CHROMagar Candida™

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Colour</th>
<th>Size (cm)</th>
<th>Form</th>
<th>Elevation</th>
<th>Margin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> ATCC 14053</td>
<td>Apple green</td>
<td>1 - 2</td>
<td>Circular</td>
<td>Convex</td>
<td>Entire</td>
</tr>
<tr>
<td><em>C. dubliniensis</em> ATCC MYA178</td>
<td>Dark green</td>
<td>0.5</td>
<td>Punctiform</td>
<td>Raised</td>
<td>Entire</td>
</tr>
<tr>
<td><em>C. rugosa</em> ATCC 10571</td>
<td>Green</td>
<td>3</td>
<td>Circular</td>
<td>Raised</td>
<td>Entire</td>
</tr>
<tr>
<td><em>C. glabrata</em> ATCC 2001</td>
<td>Lavender/Dark pink</td>
<td>3</td>
<td>Punctiform</td>
<td>Convex</td>
<td>Entire</td>
</tr>
<tr>
<td><em>C. krusei</em> ATCC 6258</td>
<td>Pink velvet</td>
<td>Irregular</td>
<td>Irregular</td>
<td>Raised</td>
<td>Filiform</td>
</tr>
<tr>
<td><em>C. tropicalis</em> ATCC 750</td>
<td>Metallic blue</td>
<td>2</td>
<td>Circular</td>
<td>Umbonate</td>
<td>Entire</td>
</tr>
<tr>
<td><em>C. parapsilosis</em> ATCC 22019</td>
<td>Pink/Light pink</td>
<td>1 - 2</td>
<td>Punctiform</td>
<td>Convex</td>
<td>Entire</td>
</tr>
</tbody>
</table>
This selective and differential medium was most effective in identifying C. glabrata which appeared as glossy lavender, C. krusei which appeared as pink velvet and C. tropicalis which appeared as metallic blue with a halo surrounding the colonies. However, the temperature and incubation period were also important in determining the identity of the clinical strains. From our observation, incubation at 30°C for 24 hours showed lighter coloured colonies or just cream colonies as seen with all the species studied except for C. krusei which appeared as pink velvet at all temperatures studied. An extension to the incubation period at this temperature still did not give clear results as there was still a mixture of cream coloured colonies or lighter shade of the colony colour as what it is supposed to be. When these clinical isolates were incubated at 35°C, the 48-hour cultures seemed to give clearer colony colours than the cultures incubated at 24 hours or 72 hours. This was the recommended temperature and period (35°C, 48 hours) by the manufacturer.

Colonies of clinical Candida species showed no variation in colour and appearance on normal agar medium (Odds & Bernaerts, 1994). However, all strains of C. dubliniensis, C. glabrata, C. krusei and C. tropicalis showed the same colony characteristics as the ATCC strains respectively. In the case of two C. albicans that exhibited cream coloured colonies, the colony characteristics were the same as the other C. albicans strains and the ATCC strain. As for C. parapsilosis one colony showed the same characteristics as C. glabrata (clinical and ATCC strain), whereas colonies of another strain of C. parapsilosis showed same characteristics as C. tropicalis (clinical and ATCC). Two species of C. rugosa showed the same colony characteristics as C. krusei and C. tropicalis.

DISCUSSION

Sensitivity of CHROMagar was 100% for C. dubliniensis (35°C and 37°C), C. tropicalis (30°C, 35°C and 37°C), C. glabrata (35°C and 37°C), C. rugosa (35°C), and C. krusei (30°C, 35°C and 37°C). It showed the least sensitivity of 14% for C. parapsilosis at 35°C. Specificity of this agar was 100% for C. albicans (30°C, 35°C and 37°C), C. dubliniensis (30°C and 35°C), C. tropicalis (30°C, 35°C and 37°C), C. glabrata (30°C and 35°C), C. rugosa (30°C and 35°C), C. krusei (30°C and 35°C) and C. parapsilosis (30°C). It had 92% specificity for C. dubliniensis at 37°C. Efficiency of CHROMagar was 100% for C. dubliniensis (35°C), C. tropicalis (30°C, 35°C and 37°C), C. glabrata (35°C), C. krusei (30°C, 35°C and 37°C) and C. rugosa (35°C). However, this agar showed only 83% sensitivity for C. parapsilosis at 35°C. Overall, it is suggestive to use CHROMagar not only for C. albicans, C. tropicalis or C. krusei as recommended by the manufacturer, but also for C. dubliniensis, C. glabrata and C. rugosa at 35°C, as recommended by the manufacturer. Our results also confirmed the results of previous investigations on the effectiveness of using CHROMagar to identify Candida species from various sources (Beighton et al., 1995; Bouchara et al., 1996; Willinger & Manafi, 1998). Oral isolates of yeasts from dental samples were identified using this medium (Beighton et al., 1995). They found distinguishable colony colours for C. albicans and C. tropicalis. They reported that C. glabrata colony colours may vary from purple to pale pink after 48 hours of incubation and may be confused with C. parapsilosis. However, they concluded that CHROMagar Candida medium was useful for the identification of oral yeast flora and recognise the presence of mixed yeast populations. In a study conducted with 6150 clinical samples, 77.5% was identified as C. albicans, 10.6% as C. glabrata, 3.8% as C. tropicalis, 2.7% as C. krusei, 2.7% as Saccharomyces cerevisiae, 2.3% as C. kefyr and the rest were either Malassezia furfur or mixed cultures using CHROMagar Candida (Bouchara et al., 1996). Willinger & Manafi (1998) evaluated the CHROMagar Candida medium for the identification of 1150 clinical isolates of yeasts. They found that 67 mixed cultures were detected using this medium, the sensitivity was 98.8% for C. albicans, 98%
for C. glabrata, 100% for C. krusei and 66.7% for C. tropicalis.

When we compared these results with the Candida cultures on potato dextrose agar (PDA), we found no difference in the colony colours for all the seven species studied as they all appeared cream. The other characteristics such as size, margin and elevation of the colonies varied, depending on the species. These colony characteristics were the same as that observed using CHROMagar Candida (Table 2). Generally all Candida colonies on agar start appearing as cream coloured colonies and gradually become darker. This was also observed by Schoofs et al. (1997) among C. albicans and C. dubliniensis isolated from HIV infected patients. Although it was reported that C. albicans can be mistaken for C. dubliniensis, but in our study the green colours for C. albicans, C. rugosa and C. parapsilosis were distinctive, which was also found by Kirkpatrick et al. (1998) and Sullivan et al. (1999). There was also distinctive identification for C. tropicalis and C. krusei especially in their colour and texture. Although C. glabrata and C. parapsilosis could be misidentified, we found that C. glabrata was glossy in texture and had a darker pink shade, i.e. lavender than C. parapsilosis. This observation was also reported by Odds & Davidson (2000). From our observation, we found that it is not necessary to have experienced laboratory personnel to identify clinical Candida species using CHROMagar if one has correct details of colony colours and characteristics for each Candida species. Thus, we would highly recommend the use of CHROMagar medium as a routine laboratory procedure to identify clinical Candida species directly from the patient. It can be used as rapid confirmation of species as an aid for effective treatment for patients with candidiasis.

CHROMagar Candida™ was found effective in our study as a primary identification method for various clinical Candida species. Since many other methods are time consuming, using CHROMagar was not only fast and easy but it was also cost effective comparatively. The significant use of this medium is to obtain the results of the culture fast enough to provide the most appropriate treatment for patients with candidiasis, as there are a few species which are resistant to certain antifungal drugs. This would avoid the usage of broad-spectrum antifungal drugs which could lead to acquired resistance among these Candida species.

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REFERENCES


