Definite differentiation of *Candida Albicans* from other species by using chrom agar

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ABSTRACT

Differences in the expression of putative virulence factors and in antifungal susceptibility among different Candida species has raised the need for species-level identification of candida. The close relationship of Candida dubliniensis with *C. albicans* has led to misidentification. Thus, the aim of the present study was to differentiate *C. albicans* among the various clinical isolates of *Candida* species. A total of 886 isolates from various clinical samples were screened for candida species and these were streaked simultaneously on CHROM agar, Pal’s agar and a combination of CHROM agar supplemented with Pal’s agar for identification. Out of 886 isolates after 48 hours of incubation on CHROM agar 75 isolates were identified as *C. albicans* produced green colonies. On Pal’s agar and CHROM agar supplemented with Pal’s agar, all 75 isolates of *C. albicans* failed to exhibit hyphal fringe. In additional test also *C. albicans* isolates grows at 45°C and failed to reduce TTC dye. CHROM agar and CHROM agar supplemented with Pal’s agar gave definitive differentiation of *C. albicans* from the yeast cultures. CHROM agar supplemented Pal’s agar makes rapid differentiation and can be easily incorporated in routine use.

Key word: *C. albicans*, CHROM agar, Pal’s agar, CHROM agar supplemented Pal’s agar

INTRODUCTION

Yeasts are now the fourth most common organisms recovered from blood culture in hospitals.[1,2] Over the past decade, there has been a significant increase in the number of reports of systemic and mucosal candida infections with non albicans candida species which were reported with nosocomial infections. The potential clinical importance has been recognized as Candida species differ in the expression of putative virulence factors and antifungal susceptibility.[3] More recently, *C. albicans* has accounted for less than 50% of blood stream infections, with increases in the frequencies of Candida glabrata, Candida parapsilosis, Candida tropicalis and other non-albicans species.[2] This transition has had significant clinical impact due to the decreased susceptibility of several non-albicans candidal yeasts to antifungal agents, specifically that of *C. glabrata* and Candida krusei to fluconazole.[4]

Changing candidal epidemiology and the availability of newer antifungal drugs with different antifungal spectrums means that physicians can no longer make therapeutic decisions based on the broad identification of fungi as either yeasts or moulds[5]; identification of candidal yeasts to the species level is now required. Identifying yeast and yeast-like organisms requires evaluation of microscopic morphology and biochemical studies.[4] Since, the various species respond differently to various newer antifungals and also in prevention of drug resistance. The initial test used by most clinical laboratories is the formation of a germ tube in serum to differentiate *C. albicans* from other yeast species.[6] This method is more time-consuming and expensive biochemical identification. [7] To fully identify yeast may take up to 72 hours from primary isolation of the organism.[8] Thus, the aim of the present study was to differentiate *C. albicans* in the various clinical isolates of Candida species.

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MATERIALS AND METHODS

We prospectively studied all clinical specimens and cultures referred to the laboratory. A total of 886 samples between August 2010 and November 2011, with universal precautions were recovered from various specimens (blood, urine, tissue and respiratory secretions) from diabetic and cancer patients and processed in the laboratory without delay. Samples were cultured on brain heart infusion agar and sabouraud dextrose agar. Identification of organisms were done by standard laboratory technique simultaneously samples were inoculated onto casein agar, pal’s agar and CHROM agar candida supplemented with pal’s agar. The following media were used for identification and differentiation of different Candida species.

CHROM agar Candida: CHROM agar Candida contains enzymatic substrates which are linked to chromogenic substrates. These substrates acted upon by different enzymes produced by Candida species. These enzymes results in colour variations which then useful for the presumptive identification of the yeasts.

Pal’s agar: Pal’s agar was freshly prepared with unsalted sunflower seeds and used within 5 days.
- Taken an aqueous extract of sunflower seeds (By pulverizing 50 g of seeds in a blender for 5 min)
- Added the ground seeds to 1 L of distilled water, followed by boiling for 30 min
- Then the seed extract was cooled and filtered and supplemented with glucose (1 g), KH2PO4 (1g) and creatinine (1 g)
- Then, the pH was adjusted to 5.5, the volume was made to 1 L and
- Before the mixture was autoclaved at 110°C for 20 min, 15 g of agar (Difco) was added
- Then medium was poured into 90-mm-diameter petridishes and cooled at (45-55°C)

CHROM agar supplemented with Pal’s agar: Equal volumes of prepared CHROM agar Candida medium and Pal’s agar were mixed for the preparation of CHROM agar Candida medium supplemented with Pal’s medium.

Additional tests for identification of C. dubliniensis and C.albicans:
1. Growth at 45°C: All C. dubliniensis and C. albicans isolates were isolated on sterile SDA and incubated at 45°C for 48-72 h. Unlike C. albicans, C. dubliniensis lacks the ability to grow at 45°C.
2. Ability to reduce 2,3,5-triphenyltetrazolium chloride (TTC): TTC is reduced by mitochondrial dehydrogenase into a water-soluble formazan product that is measured spectrophotometrically. 0.5 mL of aqueous solution (0.2% w/v) of TTC dye prepared in phosphate-buffered saline was added to the tube containing 1 mL of 0.1 optical density adjusted Candida cell culture and incubated at 37°C for 6-48 h. Unlike C. dubliniensis, C. albicans lacks the ability to metabolically reduce TTC.

Reference strain: C. albicans ATCC 10231, C. krusei ATCC 14243 and C. tropicalis ATCC 66029 were used as reference strains.

Yeast strains were cultured routinely on Sabouraud dextrose agar (SDA) and were stored in Sabouraud broth containing glycerol 30% v/v at 80°C. Before being tested on the medium, frozen yeast strains were thawed and subcultured on SDA. Yeast suspensions were then streaked to form single colonies on CAC. Inoculated plates were incubated at 37°C for 48 hours and read for up to 7 days. Plates were observed for fungal growth using morphology and colour to determine the presence of yeasts or moulds. As per the manufacturer, C. albicans, C. tropicalis, C. krusei and C. glabrata were identified by the production of green, steel blue, rough/matted/rose-coloured colonies and dark-violet respectively.

RESULTS

Specimens
From August 2010 and November 2011, 886 samples are evaluated. All the samples are plated onto media and had results available for analysis. Specimens from sterile sites included 56 cerebrospinal fluid, 42 blood and 2 catheter tip samples. Predominately non-sterile sites from which samples were submitted included respiratory secretions (178), urine (407), nail (87), scalp/hair (46) and skin (68). Respiratory samples included specimens labelled sputum, bronchoalveolar lavage, tracheal aspirate, pleural fluid and lung.
Culture results
The results were reported considering CHROM agar Candida as a primary medium for differentiating between various Candida species based on their colony colour and morphology (rough/smooth). Pal’s agar was considered as a secondary medium for differentiation.

CHROM agar Candida (CaC)
Based on the colony colour developed on CHROM agar Candida, 886 isolates of Candida species were differentiated (Table 1). The medium supported the growth of all the clinical isolates and reference strains. A wide variety of colony colours was seen, some of which were species-specific (Table 1). The white/opaque background of CAC seemed to allow good discrimination. All the *C. albicans* isolates (n = 75) formed green colonies after incubation for 48 h on CAC (Fig. 1a). This hue was distinctive for this species and, among the other species tested; only *C. dubliniensis* gave a similar colour (Fig. 1a). Isolates of *C. dubliniensis* (n = 4) developed dark bluish green colonies, and two isolates could not be distinguished from *C. albicans*. Therefore, as shown previously for CHROMagar Candida, a dark bluish green appearance on CAC may be taken as an indication of the presence of *C. dubliniensis*, but should not be used as the sole criterion for identification. All *C. krusei* (n = 2), *C. tropicalis* (n = 2) and *C. guilliermondii* (n = 4) isolates formed typical colonies that were easily differentiated from those of other yeasts. Dry, rough, pink and spreading with a dark centre (Fig. 1b). *C. tropicalis* formed dark blue colonies (Fig. 1a) and *C. guilliermondii* formed blackberry wine-coloured colonies (Fig. 1b). These findings indicate that CAC allows presumptive identification of *C. albicans*, *C. tropicalis* and *C. krusei* with high sensitivity and specificity, particularly after incubation at 37°C for 48 h. CAC can allow identification of yeasts such as *T. mucoides*, *G. capitatum* and *S. cerevisiae* was considered to be of diagnostic relevance. These species are reported increasingly as agents of invasive infections, especially in immunocompromised or critically-ill patients. Disseminated infections caused by *Trichosporon spp.* and *G. capitatum* are emerging and cause frequently fatal mycoses among neutropenic patients, particularly those who have received drugs with cytotoxic and immunosuppressive effects for haematological malignances.

Table 1: Appearance of yeast colonies after growth on Chromogenic Candida Agar for 24 and 48 h at 37°C

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of isolates</th>
<th>Isolates identified after incubation for 24/48 h</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>75</td>
<td>68/75</td>
<td>Green</td>
</tr>
<tr>
<td><em>Candida dubliniensis</em></td>
<td>4</td>
<td>3/4</td>
<td>Bluish green</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>2</td>
<td>2/2</td>
<td>Pink</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>2</td>
<td>1/2</td>
<td>Dark blue</td>
</tr>
<tr>
<td><em>Candida guilliermondii</em></td>
<td>4</td>
<td>1/4</td>
<td>Blackberry wine</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>1</td>
<td>1/1</td>
<td>Light to dark brown</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>2</td>
<td>2/2</td>
<td>Light brown</td>
</tr>
<tr>
<td><em>Candida orthopsilosis</em></td>
<td>1</td>
<td>1/1</td>
<td>Light brown</td>
</tr>
<tr>
<td><em>Candida metapsilosis</em></td>
<td>2</td>
<td>2/2</td>
<td>Light brown</td>
</tr>
<tr>
<td><em>Candida lusitanae</em></td>
<td>1</td>
<td>1/1</td>
<td>Light to dark brown</td>
</tr>
<tr>
<td><em>Candida inconspicua</em></td>
<td>0</td>
<td>N.A</td>
<td>Light brown</td>
</tr>
<tr>
<td><em>Candida norvegensis</em></td>
<td>0</td>
<td>N.A</td>
<td>Light brown</td>
</tr>
<tr>
<td><em>Candida famata</em></td>
<td>0</td>
<td>N.A</td>
<td>Light to dark brown</td>
</tr>
<tr>
<td><em>Candida kefyr</em></td>
<td>0</td>
<td>N.A</td>
<td>Light brown</td>
</tr>
<tr>
<td><em>Candida lipolytica</em></td>
<td>0</td>
<td>N.A</td>
<td>Light brown</td>
</tr>
<tr>
<td><em>Candida pelliculosa</em></td>
<td>0</td>
<td>N.A</td>
<td>Light to dark brown</td>
</tr>
<tr>
<td><em>Candida rugosa</em></td>
<td>0</td>
<td>N.A</td>
<td>Light brown</td>
</tr>
<tr>
<td><em>Candida pulcherrima</em></td>
<td>0</td>
<td>N.A</td>
<td>Light brown, pink halo</td>
</tr>
<tr>
<td><em>Geotrichum capitatum</em></td>
<td>1</td>
<td>0/1</td>
<td>Dark pink</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>0</td>
<td>N.A</td>
<td>Violet</td>
</tr>
<tr>
<td><em>Rhodotorula mucilaginosa</em></td>
<td>0</td>
<td>N.A</td>
<td>Salmon-pink</td>
</tr>
<tr>
<td><em>Trichosporon mucoides</em></td>
<td>0</td>
<td>N.A</td>
<td>Bluish green → brown</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>3</td>
<td>0/3</td>
<td>Light brown</td>
</tr>
<tr>
<td><em>Cryptococcus albidos</em></td>
<td>1</td>
<td>0/1</td>
<td>Light brown</td>
</tr>
</tbody>
</table>
Fig. 1. Appearance of yeast colonies on Chromogenic Candida Agar
(a) 1, Candida albicans; 2, Trichosporon mucoides; 3, Candida dubliniensis; 4, Candida tropicalis. (b) 1, Candida krusei; 2, Geotrichum capitatum; 3, Saccharomyces cerevisiae; 4, Candida guilliermondii. (c) 1, Candida parapsilosis; 2, Cryptococcus albidus; 3, Candida glabrata; 4, Candida lusitaniae. (d) Enlarged T. mucoides colonies.

Pal’s agar
2 out of 4 isolates of C. dubliniensis exhibited a hyphal fringe after 48 h of incubation whereas two isolates exhibited a hyphal fringe after 4 days. The 75 isolates identified as C. albicans failed to exhibit the hyphal fringe. The single Candida isolate that showed an intermittent green-coloured colony on CHROM agar produced fringe on Pal’s agar after 4 days of incubation and thus was reported as C. dubliniensis.
Figure 2: Candida dubliniensis colonies surrounded by hyphal fringe on CHROM agar supplemented with Pal’s agar

CHROM agar supplemented with Pal’s agar
2 out of the 4 isolates of C. dubliniensis that developed fringe on Pal’s agar, all isolates revealed fringe on CHROM agar supplemented with Pal’s agar (Fig. 2) whereas none of the C. albicans developed fringe on the same media.

Additional test for identification
(i) Growth at 45°C: All C. albicans isolates were found to grow at 45°C whereas all C. dubliniensis isolates failed to do so.
(ii) TTC reduction test: All C. dubliniensis isolates were able to reduce the TTC dye whereas all the C. albicans isolates failed to do so.

DISCUSSION
CHROM agar Candida had allowed the growth of most clinically relevant yeasts and also allowed presumptive identification of C. albicans, C. tropicalis, C. krusei, C. guilliermondii, and G. capitatum. CHROM agar Candida medium and Pal’s agar when used singly is time consuming whereas CHROM agar when supplemented with Pal’s agar makes rapid differentiation of both species and can be easily incorporated in routine microbiology laboratories.
CONCLUSION

CHROM agar and CHROM agar supplemented with Pal’s agar gave definitive differentiation of \textit{C. albicans} from the yeast cultures. CHROM agar when supplemented with Pal’s agar makes rapid differentiation and can be easily incorporated in routine microbiology laboratories.

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REFERENCES


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