Identification of *Candida* species using CHROM agar

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**ABSTRACT**

**Background:** *Candida* is the part of normal microbial flora of human body that potentially causes disease in the immune compromised host. Though *C. albicans* is regarded as most virulent species, non-*albicans* *Candida* are increasingly being reported in clinical samples which warrant their accurate identification that helps in directing the effective use of antifungal agents.

**Methods:** The study was done in clinical isolates of *Candida* species obtained at clinical laboratory, Nobel Hospital, Kathmandu from the period of May 2014 to April 2015. Phenotypic characterization was based on the observation of growth characteristic on Sabouraud’s Dextrose Agar, Grams’ staining, germ tube formation and characteristics appearance in CHROMagar.

**Results:** A total of 51 isolates were identified as *Candida* species. *C. albicans* (64.7%) predominated non-*albicans* *Candida* (35.3%). The majority of non-*albicans* *Candida* were *C. tropicalis*. Germ tube formation test was positive for all *C. albicans* which produced light green color in CHROM agar.

**Conclusion:** CHROM agar can be routinely used to identify different *Candida* species from clinical samples.

**Keywords:** Yeast, Phenotypic, Chromogenic

**BACKGROUND**

Medically important yeasts are abundantly present in the environment and some of these species constitute as normal flora in human beings (1). *Candida* species are commonly associated as one of the major agents causing infections in immuno-compromised patients (2). *Candida albicans* is the most virulent among the *Candida* species and is responsible for different forms of Candidiasis including infections in superficial sites and cutaneously (2). Over the last few decades, there has been an increase in the incidence of candidiasis caused by other *Candida* species (non-*albicans* *Candida*) such as *Candida dubliniensis*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, and *Candida. Parapsilosis* (3). Though molecular techniques in yeast detection are highly sensitive and specific, their implementation in routine diagnostic is limited due to complex nature of test and affordability (4). Routine characterizations of clinical isolates in resource-limited settings rely on conventionally used phenotypic methods. We aim to use a chromogenic media to characterize the *Candida* species obtained from clinical samples.

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METHODOLOGY

A hospital based descriptive cross-sectional study was conducted on the isolates of Candida species obtained in Clinical Laboratory, Nobel Hospital, Sinamangal, Kathmandu, Nepal. Clinical isolates of Candida recovered during May 2014 – April 2015 were included in the study.

For laboratory identification, samples obtained were first grown in blood culture media to rule out any contamination. Preliminary identification was done by observing growth characteristics in Sabouraud’s Dextrose Agar (SDA) slant, Grams’ staining and germ tube test. For germ tube test, a small inoculum of the test yeast cells from a pure culture was suspended in 0.5 ml human serum. The suspensions were incubated at 37 °C for three hours after which a drop of the incubated serum was placed on a microscope slide and covered with a cover-slip. Normal saline and a preparation of C. albicans ATCC 90028 was used as negative control and positive control respectively. Identification in CHROMagar plate was done by following manufacturers (HiMedia, India) instruction whereby smooth colonies that appeared light green were interpreted as C. albicans. Similarly, blue to metallic blue colored raised colonies were interpreted as C. tropicalis, likewise pink smooth colonies were considered as C. glabrata, while purple fuzzy colonies were suggestive of C. krusei as shown in Figure 1.

RESULTS

A total of 51 Candida isolates were obtained. On further speciation, the most predominant species was Candida albicans 64.7% (n=33), followed by Candida tropicalis 11.7% (n=6), Candida krusei 7.8% (n=4), Candida parapsilosis 7.8% (n=4) and Candida glabrata 7.8% (n=4) as shown in Figure 2. The presence of germ tubes was recognized as a yeast cell having about half the width and 3 to 4 times the length of the mother cell and not has the constriction at the neck of the parent cell. More than 50 % of the cells producing such a germ tubes per field were interpreted as germ tube producer Candida species. Morphologically, yeast colonies in SDA slant were white to cream colored, smooth, and glabrous. The Grams’ stain of the yeast showed Gram-positive, round-oval irregular cells which did not pose typical characteristics that could be used to differentiate them in species level. In the case of CHROM agar, the above-mentioned color profile of different colonies was used to speciate the Candida.

Table 1 depicts the findings comparing observation in CHROMagar and germ tube test. The observation for C. albicans in germ tube test and CHROM agar were consistent where the earlier could presumably be used to confirm the species. However, all other species of Candida had negative germ tube test and exhibited similar morphological characteristics in SDA agar and Grams’ staining. These species though had a different phenotypic appearance in CHROMagar.

Table 1: Phenotypic characteristics of Candida species

<table>
<thead>
<tr>
<th>Species</th>
<th>CHROM agar</th>
<th>Germ Tube Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (N=33)</td>
<td>Light green</td>
<td>33</td>
</tr>
<tr>
<td>C. tropicalis (N=6)</td>
<td>Blue purple</td>
<td>-</td>
</tr>
<tr>
<td>C. krusei (N=4)</td>
<td>Purple fuzzy</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis (N=4)</td>
<td>Pale</td>
<td>-</td>
</tr>
<tr>
<td>C. glabrata (N=4)</td>
<td>Pink smooth</td>
<td>-</td>
</tr>
</tbody>
</table>
DISCUSSION

This study has made an attempt to investigate the prevalence of different species of Candida isolated from various clinical samples submitted at the Laboratory Unit of Nobel Hospital, Kathmandu. The study was done to evaluate the performance of conventional identification phenotypic methods and CHROMagar method to identify medically important yeast in a routine clinical microbiology laboratory. Candida albicans and non-albicans Candida are increasingly being isolated from clinical specimens (5, 6). C. albicans is commonly regarded as an endogenous pathogen (7), however, the spread of this organism between patients and from hospital staffs is well recognized (8). In terms of laboratory-based identification, conventionally Candida species are identified by their growth characteristics in SDA culture media and expression of germ tube (9) which lacks in species level identification. The potential clinical importance of species-level identification has been recognized as Candida species differ in the expression of virulence factors and antifungal susceptibility (10).

CHROMagar Candida is a selective and differential medium which is widely used for the rapid identification and differentiation of Candida species from the clinical specimen. Its superiority to SDA lies on its ability to selectively inhibit bacterial growth (11). It facilitates the detection and identification of Candida species from mixed culture and provides result in 24-48 hours and has an advantage of being technically simple, rapid and cost-effective as compared to the conventional methods (12). In our study, we identified different Candida species based on color exhibited. Horvath et al. in their study described that CHROMagar Candida can readily be used in isolation of yeast from clinical specimen and reported that use of this medium allows rapid identification of clinically important Candida species (13). Similarly, Melissa and co-workers proposed that CHROMagar can only be used as principle medium to isolate yeasts from clinical samples (14). A newly described yeast, C. dubliniensis has very similar characteristics as of Candida albicans being the producer of both germ tube and chlamydosporie (15) which increases the likelihood of misidentification of the causative agent. However, CHROMagar Candida has been described to identify these two species, where after 48 hours of incubation at 37°C, C. albicans grows as light blue-green colonies as opposed to dark green colonies of C. dubliniensis (16).

C. albicans was the commonest species (64.7%) identified in our study. An increased proportion of C. albicans as compared to non-albicans Candida is reported in different instances (1, 17). In contrast, higher prevalence of non-albicans Candida (63.3% versus 36.7%) has also been reported (5, 10). Over the past decade, there has been a significant increase in the number of reports of systemic and mucosal yeast infections with both albicans and non-albicans Candida species worldwide (18). Among the non-albicans species, C. tropicalis was predominating species in our study which correlates with findings of similar studies done in Nepal (19) and elsewhere (20). Among 35.3% of non-albicans Candida, C. tropicalis (11.7%) was the most common species isolated in this study. Candida species have been found to be one of the most common pathogens causing up to 15% of nosocomial bloodstream infection and mortality rate ranging from 5-71% (21). Nevertheless in our study, no Candida species was isolated from the blood specimen.

The present study was a laboratory-based study focused on characterization of Candida species using both conventional tools and chromogenic agar based media. A further correlation of the isolated Candida species with the clinical features and the predisposing factors of each patient are warranted.

CONCLUSION

CHROMagar being relatively user friendly can routinely be used to identify different Candida species from clinical samples. Selective nature of the media and direct speciation based on color production makes it superior in terms of commonly used Sabouraud’s Dextrose Agar.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS’ CONTRIBUTION

SK designed study, performed laboratory works and drafted manuscript. PR and SG significantly contributed in designing protocol and conducting laboratory works.

REFERENCES


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