

Research Paper

Evaluation of chromogenic media and seminested PCR in the identification of *Candida* species

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Abstract

Identification of *Candida* cultured from various clinical specimens to the species level is increasingly necessary for clinical laboratories. Although sn PCR identifies the species within hours but its cost-effectiveness is to be considered. So there is always a need for media which help in the isolation and identification at the species level. The study aimed to evaluate the performance of different chromogenic media and to compare the effectiveness of the traditional phenotypic methods vs. seminested polymerase chain reaction (sn PCR) for identification of *Candida* species. One hundred and twenty seven *Candida* strains isolated from various clinical specimens were identified by conventional methods, four different chromogenic media and sn PCR. HiCrome Candida Differential and CHROMagar Candida media showed comparably high sensitivities and specificities in the identification of *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei*. CHROMagar Candida had an extra advantage of identifying all *C. parapsilosis* isolates. CHROMagar-Pal's medium identified *C. albicans*, *C. tropicalis* and *C. krusei* with high sensitivities and specificities, but couldn't identify *C. glabrata* or *C. parapsilosis*. It was the only medium that identified *C. dubliniensis* with a sensitivity and specificity of 100%. Biggy agar showed the least sensitivities and specificities. The overall concordance of the snPCR compared to the conventional tests including CHROMAgar Candida in the identification of *Candida* species was 97.5%. The use of CHROMAgar Candida medium is an easy and accurate method for presumptive identification of the most commonly encountered *Candida* spp.

Key words: chromogenic media, sn PCR, *Candida*.

Introduction

Invasive candidiasis is recognized as a leading cause of morbidity and mortality in both immunocompetent and immunocompromised critically ill patients. In intensive care unit (ICU) patients, the most common types of *Candida* infections are bloodstream, intra-abdominal, urinary tract and catheter-related infections. Until recently, *Candida albicans* was by far the predominant species, causing up to two-thirds of all cases of invasive candidiasis. However, a shift toward non-*albicans Candida* spp. (NAC) with reduced susceptibility to commonly used antifungal agents, was recently observed (Nguyen *et al.*, 1996).

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 (Hospenthal *et al.*, 2004). Prompt diagnosis of infection is of utmost importance as rapid initiation of appropriate antifungal therapy is crucial for reducing mortality (Fluit *et al.*, 2000).

Identification to the species level of yeasts cultured from various clinical specimens is increasingly necessary for clinical laboratories. Nowadays, a large variety of *Candida* spp. identification methods are commercially available, and they differ in principles, discrimination

power and cost. Generally yeast identification procedures start with a germ tube test in clinical laboratories. When the yeast cannot be identified with this method, further tests such as culturing on cornmeal agar, carbohydrate fermentation and carbohydrate assimilation tests are performed. Detection of growth patterns on cornmeal agar takes 24-72 h and sugar assimilation tests may take 72 h to two weeks. These procedures are labor intensive and take longer to determine the diagnosis and judge the proper antifungal agent (Baradkar *et al.*, 2010).

In order to facilitate rapid identification, several chromogenic substrate containing culture media have been developed. These special media yield microbial colonies with varying colors secondary to chromogenic substrates that react with enzymes secreted by microorganisms (Bernal *et al.*, 1996). Chromogenic agar carries the potential of improving identification of yeast, especially in mixed cultures (Murray *et al.*, 2005).

In order to overcome the limitations of conventional diagnostic tests, DNA-based methods have been developed for the detection of *Candida* species and offer a potentially more sensitive means of identification of the yeasts and they appear reliable and easy to use (Liguori *et al.*, 2007). DNA amplification with universal fungal primers followed by detection using species-specific probes greatly improved the sensitivity of *Candida* detection (Wahyuningsih *et al.*, 2000). This study aimed to evaluate the performance of four different chromogenic media and to compare the effectiveness and practical applicability of the traditional phenotypic methods vs. sn PCR for identification of *Candida* to the species level.

Material and Method

Clinical isolates

One hundred and twenty seven *Candida* strains were isolated from different clinical specimens from patients with nosocomial infections admitted to different ICUs of Assiut University Hospitals (in southern Egypt) during the period from October 2009 to May 2011. The *Candida* isolates were from urine (55 isolates), endotracheal aspirates (42), sputum (17), blood (5), bed sores (4), oropharyngeal specimens (2) and wounds (2).

Conventional methods

Clinical specimens were processed for the isolation of *Candida* species according to standard procedures and were identified by conventional means (McGinnis, 1994). First cultures were obtained on Sabouraud-dextrose agar (SDA) with chloramphenicol after 48 h at 37 °C. All of the yeast isolates were examined by wet mount, Gram staining and germ tube (Bhavan *et al.*, 2010), morphology and chlamyospore production on cornmeal tween 80 agar and sugar assimilation tests were done using auxanograms (Barnett *et al.*, 2000) and KB006 HiCandida Identification Kit (HiMedia, Mumbai, India) which is a combination of

12 tests for identification of *Candida* species. The plastic strip has twelve wells with sterile medium for different biochemical tests as Medium for Urease detection test, and Carbohydrate Utilization Test (with eleven different sugars in respective wells as Melibiose, Lactose, Maltos, Sucrose, Galactose, Cellobiose, Inositol, Xylose, Dulcitol, Raffinose, Trehalose).

Candida species identification on chromogenic media

Separate colonies from *Candida* isolates on SDA were subcultured on each of the following chromogenic media: HiCrome Candida Differential agar (HiMedia, Mumbai, India), CHROMagar Candida (Paris, France), CHROMagar Candida supplemented with Pal's medium and on BIGGY Agar (HiMedia, Mumbai, India). Plates were incubated at 37 °C for 24-48 h. Preparation and identification of species were performed according to the manufacturer's instructions.

Seminested PCR

Extraction of Candida DNA from culture

DNA was extracted from liquid culture of *Candida* grown for 20 - 24 h at 30 °C in YPD (1% yeast extract, 2% peptone, 2% dextrose) according to Harju *et al.* (2004). Repeated freezing-thawing of cells in a lysis buffer was used to disrupt the cell wall and release genomic DNA. Cell lysis was followed by extraction with chloroform and ethanol precipitation.

PCR primers

A 22-bp forward primer, CTSF (5-CGCATCGAT GAAGAACGCAGC-3), and a 25-bp reverse primer, CTSR (5-TCTTTTCTCCGCTTATTGATATGC-3), capable of amplifying the 3' end of 5.8S rDNA and the 5' end of 28S rDNA, including the intervening internally transcribed spacer 2 (ITS2) region, were synthesized by metabion international AG, Germany. Species-specific oligonucleotide primers for seminested PCR (snPCR) were derived from the ITS2 regions of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. dubliniensis* and are described in Table 1 (Fujita *et al.*, 1994; Ahmad *et al.*, 2002; Khan *et al.*, 2009).

DNA amplification and detection

Amplification of target DNA was carried out in thin-walled 0.2-mL PCR tubes in a total volume of 50 µL containing 1 µL AmpliTaq PCR buffer I, 1 U of AmpliTaq DNA polymerase, 10 pmol each of CTSF and CTSR primers, 1 µL of DNA extracted from culture, and 0.1 mM each deoxynucleoside triphosphate. After amplification in the first step, 1 µL of the product was further amplified using the initial reverse primer (CTSR) and a species-specific forward primer in six separate tubes corresponding to each of the *Candida* species to be detected. For snPCR, the reac-

Table 1 - Primers for species identification by snPCR.

Primer	Nucleotide sequence (5'-3')	Amplification step	Location	Amplicon size (bp)
CADET	ATTGCTTGC GCGGTAACGTCC	snPCR	ITS region of <i>C. albicans</i>	105
CPDET	ACAAACTCCAAAACCTCTTCCA	snPCR	ITS region of <i>C. parapsilosis</i>	88
CTDET	AACGCTTATTTGCTAGTGCC	snPCR	ITS region of <i>C. tropicalis</i>	106
CGDET	TAGTTTTACCAACTCGGTGT	snPCR	ITS region of <i>C. glabrata</i>	142
CKDET	GGCCGAGCGAACTAGACTTTT	snPCR	ITS region of <i>C. krusei</i>	132
CDDET	GCTAAGCGGTCTCTGGCGTCG	snPCR	ITS region of <i>C. dubliniensis</i>	100

tion mixture consisted of 1 µL AmpliTaq PCR buffer I; 1 U of AmpliTaq DNA polymerase; 5 pmol of CTSR together with 5 pmol of CADET, CPDET, CGDET, CTDET, CKDET, or CDDET; 1 µL of the first PCR product; and 0.1 mM each deoxynucleoside triphosphate. PCR cycling was carried out under the following conditions:

An initial denaturation step at 94 °C for 3 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min. with a final extension step at 72 °C for 10 min. Optimum amplification was determined to be obtained with 30 cycles of the first PCR followed by 20 cycles of the snPCR according to Ahmad *et al.* (2002). Appropriate negative controls were included in each test run, including controls omitting the DNA template during PCR assays.

Amplified DNA fragments were separated by agarose gel electrophoresis at 80 V for 1 to 2 h on gels composed of 1% (wt/vol) agarose (Sigma-Aldrich, Germany). The gels were exposed to UV light and photographed. The sizes of amplified DNA fragments were identified by comparison with molecular size marker DNA (100-bp DNA ladder).

Statistical analysis

Statistical analysis was performed using SPSS software version 16 (SPSS Inc., Chicago, USA). Values are presented as percentages of the group from which they were derived. Sensitivities and specificities of chromogenic media for the species were calculated as follows:

$$\text{Sensitivity} = \frac{\text{true positive}}{(\text{true positive} + \text{false negative})} \times 100$$

$$\text{Sensitivity} = \frac{\text{true negative}}{(\text{true negative} + \text{false positive})} \times 100$$

Percent agreement was determined by the number of isolates positive by conventional methods /number of PCR-positive isolates x 100.

Results

Candida identification by conventional methods

Among the 127 *Candida* isolates, the majority were non- *albicans* (95/127, 75%). *C. tropicalis* was the most frequent (46.5% or 59 strains) followed by *C. glabrata*

(17.4% or 22 stains). On the other hand, *C. albicans* constituted 25% of the isolates (32/127). The remaining species constituted much lower percentages as shown in Figure 1.

Candida identification on four chromogenic media

By culture on four chromogenic media, different isolated *Candida* spp. showed different colony colors and morphology which are summarized in Table 2.

The sensitivities and specificities of the chromogenic media in comparison to the conventional methods are shown in Table 3. HiCrome *Candida* and CHROMagar *Candida* media had comparably high sensitivities and specificities in identification of *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei*. CHROMagar *Candida* was the only medium that identified *C. parapsilosis* with a high sensitivity and specificity. BiGGY agar showed the lowest sensitivity and specificity in the identification of *C. albicans*, *C. tropicalis* and *C. glabrata*. CHROMagar *Candida* medium supplemented by Pal's medium was the only chromogenic medium that detected *C. dubliniensis* with 100% sensitivity and specificity.

PCR results

Out of the 127 *Candida* isolates, only 123 isolates were tested by PCR. *C. rugosa* (2 isolates) and *C. haemulonii* (1 isolate) were not tested. Regarding PCR am-

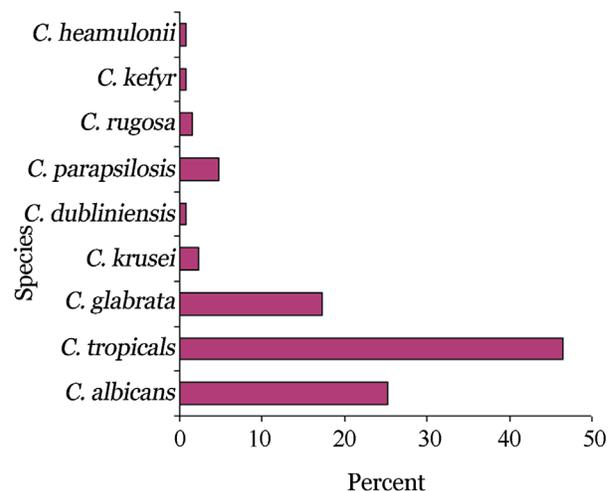


Figure 1 - Frequency of different *Candida* species identified by conventional methods.

Table 2 - Colony colours of different isolated *Candida* spp. on four different chromogenic media.

<i>Candida</i> spp. (no.)	Colony colors on different chromogenic media (no.)			
	HiCrome Candida	CHROMagar Candida	BiGGY agar	CHROMagar-Pal's
<i>C. albicans</i> (32)	Light green (32)	Light Green (31) Dark green (1)	Dark brown (23) Light brown (9)	Light green (32)
<i>C. tropicalis</i> (59)	Bluish green ^a (49) Blue purple ^a (10)	Blue (59)	Dark brown (47) Light brown (12)	Dark blue (39) Light blue (19) Light green (1)
<i>C. glabrata</i> (22)	Cream white (22)	Dark pink (22)	Creamy (19) Light brown (3)	Cream white (22)
<i>C. parapsilosis</i> (6)	Dark pink (5) Cream white (1)	White pale pink (6)	Light brown (6)	Cream white (4) Pale pink (2)
<i>C. krusei</i> (3)	Purple fuzzy ^{b,c} (3)	Pink fuzzy ^{b,c} (3)	Light brown (3)	Pink fuzzy ^{b,c} (3)
<i>C. rugosa</i> (2)	Light blue ^c (2)	Light green ^c (1) Light blue ^c (1)	Light brown (2)	Light blue ^c (2)
<i>C. dubliniensis</i> (1)	Light green (1)	Light green (1)	Dark brown (1)	Dark green ^d (1)
<i>C. kefyr</i> (1)	Greenish brown (1)	White pale pink (1)	Light brown (1)	Pale pink (1)
<i>C. haemulonii</i> (1)	Greenish brown (1)	White pale pink (1)	Light brown (1)	Pale pink (1)

a. Halo which diffused into surrounding agar, b. Large rough, c: flat pale edges, d: rough with fringe.

plification of *Candida* rDNA with universal fungal primers, the first round of PCR amplification of rDNA was performed with genomic DNA from different *Candida* spp. and non-fungal organisms with CTSF and CTSR primers and the amplified products were detected by agarose gel electrophoresis (AGE). The PCR amplification with these two primers and genomic DNA from *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. dubliniensis*, and *C. parapsilosis* resulted in amplification of DNA fragments of 344, 334, 423, 335, 337 and 317 bp respectively (Figure 2).

In sn PCR, reamplification of the product of the first PCR with CTSR and the species-specific primers corresponding to the ITS2 sequences from *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. dubliniensis*, and *C. parapsilosis* resulted in specific amplification of single DNA products of the expected sizes (105, 106, 142, 132, 100, 88 bp respectively) (Figure 3).

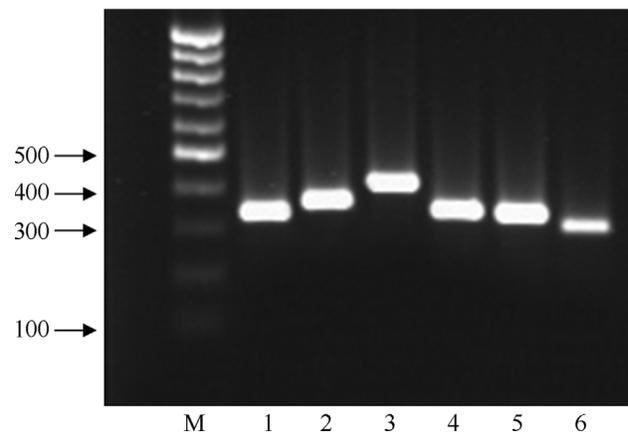


Figure 2 - PCR amplification of genomic DNAs of 6 *Candida* spp. with universal fungal primers. Lane M: 100-bp molecular size marker, Lane 1: *C. tropicalis*, Lane 2: *C. albicans*, Lane 3: *C. glabrata*, Lane 4: *C. krusei*, Lane 5: *C. dubliniensis*, and Lane 6: *C. parapsilosis*.

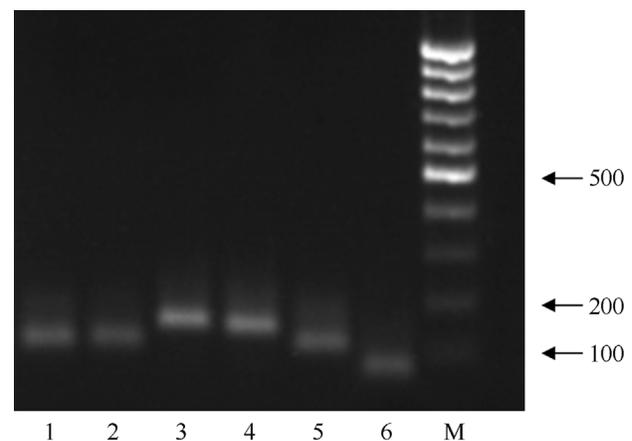


Figure 3 - Lanes 1 to 6, snPCR amplification of DNAs from *C. tropicalis*, *C. albicans*, *C. glabrata*, *C. krusei*, *C. dubliniensis* and *C. parapsilosis* respectively, using primer CTSR with primers CTDET, CADET, CGDET, CKDET, CDDDET, and CPDET respectively. Lane M, 100-bp molecular size marker.

Table 3 - Sensitivity and specificity of the chromogenic media in relation to the conventional phenotypic methods.

<i>Candida</i> spp. (no)	HiCrome Candida		CHROMagar Candida		BiGGY agar		CHROMagar-Pal's	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
<i>C. albicans</i> (32)	100	98.9	96.9	97.9	71.9	50.5	100	100
<i>C. tropicalis</i> (59)	100	100	100	98.5	79.7	66.1	100	97
<i>C. glabrata</i> (22)	100	99	100	100	86.4	100	-	-
<i>C. parapsilosis</i> (6)	-	-	100	98.3	-	-	-	-
<i>C. krusei</i> (3)	100	100	100	100	-	-	100	100
<i>C. rugosa</i> (2)	-	-	-	-	-	-	-	-
<i>C. dubliniensis</i> (1)	-	-	-	-	-	-	100	100

The results of snPCR in species identification were concordant with those of the conventional phenotypic methods for all isolates of *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. dubliniensis* species. But for the 3 isolates of *C. parapsilosis*, there was discrepancy between snPCR and the phenotypic tests. Thus, the overall concordance of the snPCR compared to the conventional phenotypic tests in the identification of *Candida* species was found to be 97.5% (120 of 123) as shown in Table 4.

Discussion

Rapid presumptive identification of yeast species is quite difficult in resource- limited countries due to the lack of training, proper reagents and equipments. 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 time required for these tests which increase the cost of mycology cultures (Pfaller *et al.*, 1996). As a result, direct selection of appropriate agents for antifungal therapy or prophylaxis becomes almost impossible. That's why there is always a need for media which help not only in the isolation but also in the identification at the species level (Nadeem *et al.*, 2010).

Table 4 - Agreement between the conventional phenotypic methods and sn PCR in the identification of *Candida* species.

<i>Candida</i> spp.	No. of isolates identified by:		% agreement
	Conventional phenotypic methods	snPCR	
<i>C. albicans</i>	32	32	100
<i>C. tropicalis</i>	59	59	100
<i>C. glabrata</i>	22	22	100
<i>C. parapsilosis</i>	6	3	50
<i>C. krusei</i>	3	3	100
<i>C. dubliniensis</i>	1	1	100
Total	123	120	97.5

Chromogenic media have the advantage of rapid identification of *Candida* species, technically simple preparation (by boiling), rapid and cost effective compared to technically demanding time consuming and expensive conventional method (Vijaya *et al.*, 2011). In this study, the colony appearance of 127 *Candida* isolates on four chromogenic media was compared in order to identify the most easy and reliable one for rapid identification of different *Candida* species. Compared to the results of the conventional methods, the sensitivity and specificity of HiCrome Candida Differential agar (HiMedia, Mumbai, India) were determined as 100% and 98.9% for *C. albicans*, 100% and 100% for *C. tropicalis*, 100% and 99% for *C. glabrata*, and 100% and 100% for *C. krusei*.

Regarding *C. albicans*, our results are comparable to many previous studies. Willinger *et al.* (2001) reported 98.8% sensitivity and 100% specificity. Baradkar *et al.* (2010) reported a sensitivity and specificity of HiChrom Candida agar to be 96.55 and 96.42% respectively. Yucesoy and Marol (2003) reported the sensitivity and specificity to be 99.4% and 100% respectively.

For *C. tropicalis*, similar results were previously reported by Yucesoy and Marol (2003). Baradkar *et al.* (2010) found the sensitivity and specificity to be 100%. On the other hand, Peng *et al.* (2007) showed 100% sensitivity and 78.8% specificity.

We couldn't distinguish *C. dubliniensis* from *C. albicans* isolates as we had only one isolate that gave light green colored growth so it is difficult to evaluate HiChrom Candida agar for the identification of this species.

For *C. parapsilosis*, 83% of the isolates produced pink colonies while 17% produced cream colonies similar to those of *C. glabrata*. As *C. glabrata* doesn't produce pseudohyphae on corn meal/Tween 80 agar, combination of the two media can distinguish the two species within 48 h as compared to 48 h to 2 weeks required for sugar assimilation tests (Willinger *et al.*, 2001). These results were similar to those reported by Girgis *et al.* (2009) and Vijaya *et al.* (2011). We reported a sensitivity and specificity for identification of *C. glabrata* to be 100% and 99% respectively comparable to Willinger *et al.* (2001) but higher than other studies (Yucesoy and Marol, 2003; Peng *et al.*, 2007;

Baradkar *et al.*, 2010). The results in this study concerning *C. parapsilosis* differed from Baradkar *et al.* (2010) who described that the majority of growth gave cream colored colonies (8/10) while the minority (2/10) produced pink colonies. *C. rugosa* produced characteristic easily identifiable colony color and morphology on HiCrome Candida Differential agar. Other *Candida* species (*C. kefyr* and *C. haemulonii*) could not be differentiated from each other on this medium as both produced greenish brown colonies. Unfortunately, we couldn't find any other reports evaluating HiCrome Candida Differential agar (HiMedia, Mumbai, India) for identification of these less common *Candida* species.

The sensitivity and specificity of CHROMagar Candida (Paris, France) were determined as 96.9% and 97.9% for *C. albicans*, 100% and 98.5% for *C. tropicalis*, 100% and 100% for *C. glabrata*, 100% and 100% for *C. krusei*, and 100% and 98.3% for *C. parapsilosis* respectively. *C. rugosa* appears to typically produce a readily identifiable and unique color/colony type on CHROMagar Candida as previously reported (Hospenthal *et al.*, 2006). In agreement, Nadeem *et al.* (2010) reported that CHROMagar Candida correctly identified the majority of *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei* with comparable high sensitivities and specificities.

Regarding *C. glabrata*, we didn't agree with Hospenthal *et al.* (2006), who reported that they couldn't distinguish *C. glabrata* from others that gave pink to lavender coloured colonies. *C. kefyr* and *C. haemulonii* could not be differentiated from *C. parapsilosis* on CHROMagar Candida. Also, *C. dubliniensis* could not be distinguished from *C. albicans* green colonies. These results were consistent with those of Hospenthal *et al.* (2006) and Nadeem *et al.* (2010).

The addition of Pal's agar to CROMagar Candida medium facilitated the differentiation of *C. dubliniensis* from *C. albicans* with 100% sensitivity and specificity for both species, where *C. albicans* isolates appeared as light green smooth colonies while *C. dubliniensis* showed distinguishable dark green rough colonies which were accompanied by the production of chlamyospores. These results were in accordance with those of Sahand *et al.* (2005). Other *Candida* species that were assayed in this study showed rough or smooth colonies in CHROMagar Candida supplemented with Pal's agar; however, they never developed green colonies nor produced chlamyospores, making their misidentification as *C. albicans* or *C. dubliniensis* highly unlikely. Interestingly, *C. tropicalis* and *C. krusei* retained a distinguishable blue and pink-and-rough colony aspect, respectively, that allowed their easy identification on CHROMagar Candida medium supplemented with Pal's agar with sensitivity and specificity detected as 100% and 97% for *C. tropicalis* and 100% for *C. krusei* respectively. On the other hand, *C. glabrata*, *C. parapsilosis*, *C. kefyr* and *C. haemulonii* produced indistinguishable pale pink to

cream white smooth colonies making it difficult to identify them on CHROMagar Candida supplemented with Pal's medium. These results were in harmony with Sahand *et al.* (2005).

The sensitivity and specificity of BiGGY agar were detected as 71.9% and 50.5% for *C. albicans*, 79.7% and 66.1% for *C. tropicalis*, and 86.4% and 100% for *C. glabrata* respectively. It was difficult to differentiate the colors of *C. albicans* and *C. tropicalis* colonies on this medium. In the present study, most *C. glabrata* strains (86.4%, 19/22) showed distinctive appearance on BiGGY agar after 48 h. Brown color was only observed at the first streaks of the cultures, where the colonies were very crowded, while other areas especially single colonies were very small and creamy colored. These data agreed with those of Yücesoy and Marol (2003) with the exception of *C. krusei* which they reported to show typical distinctive appearance on BiGGY agar with 100% sensitivity and specificity. The discrepancy may be due to the small number of *C. krusei* (3 isolates) tested in our study compared to 12 isolates in the study of Yücesoy and Marol (2003). The lower sensitivity and specificity of BiGGY agar to identify commonly isolated *Candida* species potentially limits the clinical usefulness of this agar despite its low cost compared to other chromogenic media.

As Culture-based phenotypic identification techniques are slow and especially prone to misidentification of fungal pathogens. Numerous DNA-based methods have developed to diagnose *Candida* infections. In the present study, snPCR assays targeting species-specific sequences in the rDNA have been used for the specific detection of six clinically important *Candida* species. We used the sn PCR amplification method according to many previous studies (Ahmad *et al.*, 2002, 2004; Çerikçioğlu *et al.*, 2010). The reamplification step introduced has resulted in maximum sensitivity of the assay with a specificity of 100% (Ahmad *et al.*, 2004; Çerikçioğlu *et al.*, 2010).

The target for snPCR amplification was rDNA which is considered to be most suitable for sensitive detection of *Candida* because it is present in multiple copies (50 to 100 copies) per *Candida* genome (Reiss *et al.*, 1998). Moreover, between the highly conserved rDNA subunits are the internally transcribed spacers, which contain sequences unique to each *Candida* species, and thus the use of primers corresponding to these regions facilitates species identification (Ahmed *et al.*, 2002).

The results presented in this study showed a 97.5% overall concordance of the snPCR with the conventional methods including the use of CHROMagar Candida medium in the identification of *Candida* species which were comparable to those of Ahmad *et al.* (2002) and Girgis *et al.* (2009). Sahiner *et al.* (2011) reported that the traditional phenotypic methods including CHROMagar Candida medium yielded the same results (100% compatible) as PCR in the species identification of *Candida* isolates.

The discrepancy we found was among 3 isolates which were phenotypically identified as *C. parapsilosis* but they showed negative results when tested with all species-specific primers used in the study (50%). Although the precise reason for this discrepancy remains unclear, it could be related to inadequacy of the presently available phenotypic *Candida* identification methods. Further confirmation by DNA sequencing may be required to correctly identify these isolates. Ahmad *et al.* (2004) reported a closely related finding. They found that no amplification was detected in snPCR from *C. krusei*, *C. lusitaniae* and *C. dubliniensis*.

The snPCR used in this study has an average processing time of 9 to 10 h, does not require the use of hybridization probes and radioactive substances, and is specific. The snPCR appears to be at least 100 times more sensitive than the multiplex PCRs (Ahmed *et al.*, 2002).

In conclusion, CHROMagar *Candida* medium (CHROMagar Company, Paris, France) is an easy and accurate method for presumptive identification of the most commonly encountered *Candida* spp. (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei*) within 48 h. Although sn PCR identified the species within 8-10 h but its cost-effectiveness should also be considered. So these phenotypic methods should be considered in the identification of *Candida* species in areas with limited resources.

References

- Ahmad S, Khan Z, Mustafa AS, Khan ZU (2002) Seminested PCR for diagnosis of candidemia: comparison with culture, antigen detection, and biochemical methods for species identification. *J Clin. Microbiol.* 40(7):2483-2490.
- Ahmad S, Mustafa AS, Khan Z, Al-Rifaiy AI, Khan ZU (2004) PCR-enzyme immunoassay of rDNA in the diagnosis of candidemia and comparison with amplicon detection by agarose gel electrophoresis. *Int. J Med Microbiol* 294:45-51.
- Baradkar VP, Mathur M, Kumar S (2010) Hichrom *Candida* agar for identification of *Candida* species. *Indian. J. Pathol. Microbiol* 53(1):93-94.
- Barnett JA, Pane RW, Yarrow D (2000) *Yeasts: Characteristics and Identification*. Cambridge University Press: Cambridge, 3rd edition, pp 26-27.
- Bernal S, Mazuelos ME, Garcia M, Aller AI, Martinez MA, Gutierrez MJ (1996) Evaluation of CHROMagar *Candida* medium for the isolation and presumptive identification of species of *Candida* of clinical importance *Diagn Microbiol Infect Dis* 24(4):201-204.
- Bhavan PS, Rajkumar R, Radhakrishnan S, Seenivasan C, Kannan S (2010) Culture and Identification of *Candida albicans* from Vaginal Ulcer and Separation of Enolase on SDS-PAGE. *Interna J Biol* 2:84-94.
- Çerikçioğlu N, Aksu B, Dal, TD, Deniz U, Bilgen HS, Özek E, Söyletir G (2010) Seminested PCR for detection and identification of *Candida* species directly from blood culture bottles. *New. Microbiologica.* 33:57-62.
- Fluit AC, Jones ME, Schmitz FJ, Acar J, Gupta R, Verhoef J (2000) Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY antimicrobial surveillance program, 1997 and 1998. *Clin Infect Dis* 30(3):454-460.
- Fujita S, Lasker BA, Lott TJ, Reiss E, Morrison CJ (1995) Micro-titration plate enzyme immunoassay to detect PCR-amplified DNA from *Candida* species in blood. *J Clin Microbiol* 33:962-967.
- Girgis SA, El-Mehalawy AA, Rady LM (2009) Comparison between culture and non-culture based methods for detection of Nosocomial fungal infections of *Candida* spp. in intensive care unit patients. *Egypt. Acad. J. Biolog. Sci.* 1:37-47.
- Harju S, Fedosyuk H, Peterson KR (2004) Rapid isolation of yeast genomic DNA: Bust n' Grab. *BMC Biotechnol* 4:8.
- Hospenthal DR, Beckius ML, Floyd KL, Horvath LL, Murray CK (2006) Presumptive identification of *Candida* species other than *C. albicans*, *C. krusei*, and *C. tropicalis* with the chromogenic medium CHROMagar *Candida*. *Ann Clin Microbiol Antimicrobiol* 3:5-1.
- Hospenthal DR, Murray CK, Rinaldi MG (2004) The role of antifungal susceptibility testing in the therapy of candidiasis. *Diagn Microbiol Infect Dis* 48(3):153-160.
- Khan Z, Mustafa AS, Alam FF (2009) Real-time LightCycler polymerase chain reaction and melting temperature analysis for identification of clinically important *Candida* spp. *J Microbiol Immunol Infect* 42:290-295.
- Liguori G, Lucariello A, Colella G, De Luca A, Marinelli P (2007) Rapid identification of *Candida* species in oral rinse solutions by PCR. *J Clin Pathol* 60:1035-1039.
- McGinnis MR (1994) Mycology. In Isenberg H. D. (ed.). *Clinical microbiology procedure handbook*. American Society for Microbiology, Washington D.C., pp 6.1-6.12.
- Murray CK, Beckius ML, Green JA, Hospenthal DR (2005) Use of chromogenic medium for the isolation of yeasts from clinical specimens. *J Med Microbiol* 54:981-985.
- Nadeem, S.G.; Hakim, S.T.; Kazmi, S.U. (2010). Use of CHROMagar *Candida* for the presumptive identification of *Candida* species directly from clinical specimens in resource-limited settings. *Libyan J Med.* 5:2144-2150.
- Nguyen MH, Peacock JE, Morris A J, Tanner DC, Nguyen ML, Snyderman DR, Waqener MM, Rinaldi MG, Yu VL (1996) The changing face of candidemia: emergence of non-*Candida albicans* species and antifungal resistance. *Am. J Med* 100(6):617-623.
- Peng, C.F.; Lee, K.M.; Lee, S.H. (2007). Characterization of two chromogenic media of *Candida* ID₂ and CHROMagar *Candida* for preliminary identification of yeasts. *J Biomed Lab Sci* 19:63-68.
- Pfaller MA, Houston A, Coffmann S (1996) Application of CHROMagar *Candida* for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida (Torulopsis) glabrata*. *J Clin Microbiol* 34(1):58-61.
- Reiss E, Tanaka K, Bruker G, Chazalet V, Coleman D, Debeauvais JP, Hanazawa R, Latge JP, Lortholary J, Makimura K, Morrison CJ, Murayama SY, Naoe S, Paris S, Sarfati J, Shibuya K, Sullivan D, Uchida K, Yamaguchi H (1998) Molecular diagnosis and epidemiology of fungal infections. *Med Mycol* 36:249-256.

- Sahand IH, Moragues MD, Eraso E, Villar-Vidal M, Quindos G, Ponton J (2005). Supplementation of CHROMagar *Candida* medium with Pal's medium for rapid identification of *Candida dubliniensis*. *J Clin Microbiol* 43 (11):5768-5770.
- Sahiner F, Ergünay K, Ozyurt M, Ardyç N, Hosbul T, Haznedaroglu T (2011) Phenotypic and genotypic identification of *Candida* strains isolated as nosocomial pathogens. *Mikrobiyol Bul* 45:478-488.
- Vijaya D, Harsha TR, Nagaratnamma T (2011) *Candida* Speciation Using Chromagar. *J Clin Diagn Res* 5:755-757.
- Wahyuningsih R, Freisleben HJ, Sonntag HG, Schnitzler P (2000) Simple and rapid detection of *Candida albicans* DNA in serum by PCR for diagnosis of invasive candidiasis. *J Clin Microbiol* 38:3016-3021.
- Willinger B, Hillwoth C, Selitsch B, Manafi M (2001) Performance of Candida ID, a new chromogenic medium for presumptive identification of *Candida* species, in comparison to CHROMagar *Candida*. *J Clin Microbiol* 39(10):3793-3795.
- Yucesoy M, Marol S (2003) Performance of CHROMagar *Candida* and BIGGY agar for identification of yeast species. *Ann Clin Microbiol Antimicrobiol* 2:1-8.

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