Original Articles

Performance of Chromogenic Candida Agar and CHROMagar Candida in recovery and presumptive identification of monofungal and polyfungal vaginal isolates

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Chromogenic Candida agar (OCCA) is a novel medium facilitating isolation and identification of Candida albicans, C. tropicalis, and C. krusei, as well as indicating polyfungal population in clinical samples. We compare the performance of OCCA, to CHROMagar Candida (CAC) and Sabouraud chloramphenicol agar (SCA). Vaginal swab samples from 392 women were simultaneously inoculated onto three study media. A total of 161 (41.1%) were found to be positive for fungi of which 140 (87%) were monofungal, and 21 (13%) polyfungal. One-hundred and fifty-seven samples (97.5%) were positive on CAC, 156 (96.9%) on OCCA, 148 (91.9%) on SCA and 144 (89.4%) samples were positive on all three media. The yeasts were identified by conventional methods including germ tube test, microscopic morphology on cornmeal-Tween 80 agar, and the commercial API 20C AUX. The 182 isolates were C. albicans (n = 104), C. glabrata (n = 51), C. krusei (n = 7), C. tropicalis (n = 5), C. famata (n = 3), C. kefyr (n = 3), C. zeylanoides (n = 3), C. colliculosa (n = 2), and other species of Candida (n = 4). Among the 21 polyfungal populations, 20 (95.2%) were detected in OCCA, 14 (66.7%) in CAC, and 13 (61.9%) in CAC and OCCA (P < 0.05). Most polyfungal populations (47.6%) yielded C. albicans + C. glabrata. The efficiency of both chromogenic media for C. albicans was ≈92.9% at 72 h. OCCA is more efficient and reliable for rapidly identifying C. albicans and polyfungal populations than CAC. However, CAC is more efficient for identifying C. krusei and C. tropicalis.

A chromogenic agar with a higher isolation rate of yeasts and better detection of polyfungal populations than SCA, is suggested as a medium of first choice when available.

Keywords Candida albicans, Chromogenic Candida agar, CHROMagar Candida, identification, vagina

Introduction

Vulvovaginal candidosis (VVC) is one of the most common superficial mycoses. Three out of four women experience VVC at least once, while one in two get it twice or more during their lifetime [1]. Candida albicans is the causative agent in 80–90% of all cases, while it is C. glabrata in 2–10%, and C. krusei in 1–3%, followed by C. tropicalis, C. kefyr, C. parapsilosis, and other species of the genus [1,2]. It is generally not justified to conduct antifungal susceptibility testing in gynecological yeast
infections [2]. However, identification of the specific Candida species is important to initiate and guide appropriate therapy [1]. The azole susceptibility of C. albicans is well-known, and supports the continued use of azole agents (i.e., clotrimazole, miconazole, or fluconazole) for empirical therapy of uncomplicated VVC [3]. On the other hand, isolates of C. krusei exhibit innate resistance to fluconazole, and C. glabrata can be susceptible or resistant to this triazole, depending on the dose [2,3].

In the last decade, advances have been made in laboratory methods for diagnosis of Candida species, especially C. albicans, resulting in more rapid and reliable identification. One of these methods is the incorporation of fluorogenic or chromogenic substrates directly into the growth agar media. This advance provides rapid and presumptive identification of Candida species on the primary isolation medium. It also reveals species-specific enzyme activity, which allows an easier discrimination of Candida species in polyfungal populations than does the traditional medium, Sabouraud agar. These media may avoid or diminish the need for subculture and further biochemical testing, which considerably simplifies the identification procedure [4,5]. Because of these advantages, several chromogenic media have been produced and introduced into routine laboratory practice [6–8].

CHROMagar Candida (CAC, Becton Dickinson, Heidelberg, Germany) is a differential ready-to-use chromogenic medium designed to identify C. albicans, C. tropicalis, and C. krusei by colony color and morphology. Colonies of C. albicans appear light to medium green; C. tropicalis colonies appear dark blue to metallic blue; and C. krusei pink with a whitish border. Other yeasts may develop either light to dark mauve or cream colors (e.g., C. glabrata) on isolation media [9,10]. Chromogenic Candida agar (OCCA, Oxoid, Basingstoke, UK) contains a chromogenic substratum for rapid detection and specific identification of such Candida spp. Typically, on OCCA, colonies of C. albicans are green; C. tropicalis are dark blue; and pink, irregular and dry colonies indicate C. krusei. OCCA permits the identification of C. guilliermondii, which forms blackberry wine-colored colonies [11]. Other Candida species produce bluish-green, beige, yellow, maroon, or violet colonies [6,11].

This study evaluates two of these chromogenic media, OCCA and CAC, to (i) compare recovery, (ii) assess the accuracy of presumptive species based on colony characteristics, and (iii) elucidate the presence of polyfungal populations in vaginal samples.

Material and methods

Study population

Between April 2006 and May 2006, 392 patients who applied to the outpatients clinics of the Cukurova Statal Gynecology-Obstetrics and Children’s Hospital were included in this study.

Clinical samples

Every participant, with several symptoms, e.g., itching, burning, soreness, irritation and/or whitish discharge, provided a single vaginal swab sample. Samples were taken by sterile swabs using a dry sterile speculum from the lateral vaginal wall, and inoculated into 1 ml of Sabouraud 4% glucose broth for transport to the laboratory. Samples were kept in this broth for a maximum of 30 min before inoculation of plates by subculturing the swab.

Commercial media

Sabouraud glucose agar (Difco Laboratories, Detroit, MI, USA) and OCCA were purchased as powdered media and prepared according to the manufacturer’s instructions, in Petri dishes containing 20 ml of the liquid medium, amended with 50 mg/liter chloramphenicol (Fluka, BioChemika, China). All plates were stored at 4°C and equilibrated to room temperature prior to inoculation.

Evaluation of the media

All samples were simultaneously inoculated by streaking OCCA, CAC, and SCA. After inoculation, the SCA and CAC cultures were incubated at 37°C in ambient air, while OCCA plates were incubated at 30°C, according to the manufacturer’s instructions. Interpretation of the results was carried out independently by two of the authors, after 24, 48, and 72 h of incubation.

Fungal identification

For each medium, we noted the following parameters: number of positive cultures, morphology and pigmentation of the colonies, the presence of polyfungal cultures indicated by phenotypically different colonies, and the final identities of all isolates. The isolates were analyzed macroscopically according to the manufacturers’ recommendations and were then identified by conventional methods including germ tube test (GTT) in human serum at 37°C for 2 h; microscopic morphology on cornmeal-Tween 80 agar (CTA; Difco, Detroit,
The colony appearances of \( C. \) albicans on OCCA, CAC, and SCA were compared, and analyzed in terms of sensitivity, number of true positives/(number of true positives + number of false negatives); and specificity, number of true negatives/(number of true negatives + number of false positives); positive predictive value (PPV), number of true positives/(number of true positives + number of false positives); negative predictive value (NPV), number of true negatives/(number of true negatives + number of false negatives); and efficiency, (number of true positives + number of true negatives)/(number of true positives + number of true negatives + number of false positives + number of false negatives) using the quality controls described above. McNemar \( \chi^2 \) test was used in the dependent groups regarding the comparison of CAC and OCCA. A \( P \) value of <0.05 was considered statistically significant.

Results
No growth was obtained on any media from 231 specimens (58.9%). However, 161 specimens (41.1%) yielded yeasts, with 157 (97.5%) positive on CAC, 156 (96.9%) on OCCA, and 148 (91.9%) on SCA, while 144 (89.4%) clinical specimens were positive on all three media. Mono- fungal and polyfungal populations were detected in 140 (86.9%) and 21 (13.1%) cultures, respectively. \( C. \) albicans was the most frequent species in 104 isolates (57.1%) followed by \( C. \) glabrata in 51 (28%), \( C. \) krusei in 7 (3.8%), \( C. \) tropicalis in 5 (2.8%), \( C. \) famata in 3 (1.6%), \( C. \) kefyr in 3 (1.6%), \( C. \) zeylanoides in 3 (1.6%), \( C. \) colliculosa in 2 (1.1%), \( C. \) guilliermondii in 1 (0.6%), \( C. \) rugosa in 1 (0.6%), \( C. \) sphaerica in 1 (0.6%), and \( C. \) utilis in 1 (0.6%). The distribution of 182 yeast isolates on CAC and OCCA in terms of colony color at 72 h are illustrated in Table 1.

Among 104 \( C. \) albicans isolates, 73 (70.2%), 88 (84.6%), and 91 (87.5%) produced expected green colonies on CAC at 24, 48 and 72 h, respectively, while 89 (85.6%), 95 (91.3%) and 97 (93.2%) on OCCA at the same periods of time. At the end of 72 h, four of \( C. \) albicans strains presented white, and one lavender colonies on CAC, while four \( C. \) albicans strains were beige on OCCA. However, eight (7.7%) isolates of \( C. \) albicans failed to grow on CAC, and 3 (2.9%) on OCCA. Sensitivity, specificity, PPV, NPV, and efficiency values of \( C. \) albicans on CAC and OCCA plates at 72 h are shown in Table 2.

The expected dark blue colony formation with a lavender halo on CAC could be found with only three out of five (60%) \( C. \) tropicalis isolates at 24 h, with no change at 48 or 72 h. On CAC, 5 out of 7 (71.4%) \( C. \) krusei isolates were colored

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colonies produced expected dry pink color at 24 h, and 6 (85.7%) at 72 h. However, no production of characteristic colonies was observed on OCCA by either \textit{C. tropicalis} or \textit{C. krusei} at any time. The rest of the colonies of non-\textit{C. albicans} \textit{Candida} species did not present any specific color.

Among the 21 polyfungal populations, 20 (95.2%) were detected on OCCA, and 14 (66.7%) on CAC (\(\chi^2\text{McNemar} = 4.5, \text{df} = 1, P < 0.05\)) (Table 3). Bacterial contamination was minimal on CAC and OCCA plates (<1%).

### Discussion

The performance and efficiencies of chromogenic media in identifying \textit{Candida} species from female genital samples have been widely compared and discussed in the literature. Various media, such as Pagano-Levin agar [14,15], Biggy [14,16], CAC [3,7,8,14,17,18], Albicans ID [8,16] and CandiSelect [8] have been studied. The choice of a particular chromogenic medium depends on the origin of the clinical samples (percentage of polyfungal vs. monofungal cultures), the distribution of species (percentage of \textit{C. albicans}, \textit{C. tropicalis}, and \textit{C. krusei}), and the cost of the medium [8]. In most reports, chromogenic media were effective for isolating fungi and identifying \textit{Candida} species, especially \textit{C. albicans}, and for the detection of polyfungal populations from clinical samples, particularly vaginal swabs [7,8,14–18]. Another advantage of these chromogenic media was their ease of use in the identification of non-\textit{C. albicans} \textit{Candida} species, 24–48 h earlier than traditional media [6–8,11,14,16–18]. However, as expected and observed, performances of various chromogenic media differ from each other. They also differ over time, because they are subject to continuous development.

Recently, Silva \textit{et al.} [14] suggested that more than one culture medium should be used for an adequate primary isolation. In this present investigation, the isolation rates of CAC (97.5%) and OCCA (96.9%) were found to be rather similar, and better than SCA (91.9%). More recently, we detected that the chromogenic media Albicans ID2 and Biggy agar were not sufficient for reliable differentiation of yeasts to the species level, requiring additional classical differentiation methods, (e.g., subcultivation on CTA, assimilation techniques such as ID 32C) to identify clinical yeast isolates [16]. However, the chromogenic medium, CAC, has usually been found to provide preliminary differentiations of \textit{C. albicans}, \textit{C. tropicalis}, and \textit{C. krusei} with high levels of sensitivity and specificity [6–10, 14, 17–22].

In this study, we determined that OCCA provided better identification of \textit{C. albicans} than CAC at 24 h (85.6% vs. 70.2%), and at 72 h (93.2% vs. 87.5%), respectively (Table 2). We observed that 5 isolates of \textit{C. albicans} were misidentified as non-\textit{C. albicans} \textit{Candida} species, because four of their colonies were white and one was lavender on CAC with four being beige on OCCA at 72 h (Table 1). On CAC, three of five \textit{C. tropicalis} strains were identified and they produced lavender colonies at 24 h. However, lavender colony formation by several species of \textit{Candida}, including \textit{C. albicans}, but especially \textit{C. glabrata}, was detected on CAC. Also, six of seven \textit{C. krusei} isolates had distinctive appearance with their fuzzy, rough, large and pale pink colonies with matte surfaces on CAC at 72 h, as described in other studies [2,7,22,23]. However, OCCA did not identify three \textit{C. tropicalis} or six \textit{C. krusei} strains which were appropriately identified by CAC. Additionally, it did not present any distinctive appearance for other non-\textit{C. albicans} \textit{Candida} species. Moreover, one isolate of \textit{C. tropicalis} produced a green color instead of the expected dark blue color on OCCA (Table 1).

Several reports noted that \textit{C. glabrata} can be distinguished from other \textit{Candida} spp. by the production of pale pink to lavender colonies on CAC [19–23]. Others did not concur, finding, as we did, that \textit{C. glabrata} strains showed significant variability [6,8–10,18,24]. Recently, Hospenthal \textit{et al.} [20] reported that most non-\textit{C. albicans} \textit{Candida} species are pink, lavender, or ivory on CAC. It was also noted that \textit{C. lipozytica}, \textit{C. norvegensis}, \textit{C. firmetaria}, and \textit{C. inconspicua} strains may not be distinguishable from \textit{C. krusei} colonies by their colors and morphologies [10,20]. Hospenthal \textit{et al.} [20] described that most \textit{C. rugosa} strains produced a

### Table 2  
Sensitivity, specificity, PPV, NPV, and efficiency values of \textit{Candida albicans} on CAC and OCCA plates at 72 h.

<table>
<thead>
<tr>
<th>Value</th>
<th>CAC (%)</th>
<th>OCCA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>87.5</td>
<td>93.2</td>
</tr>
<tr>
<td>Specificity</td>
<td>100</td>
<td>98.7</td>
</tr>
<tr>
<td>PPV</td>
<td>100</td>
<td>99.0</td>
</tr>
<tr>
<td>NPV</td>
<td>85.7</td>
<td>91.7</td>
</tr>
<tr>
<td>Efficiency</td>
<td>92.9</td>
<td>95.6</td>
</tr>
</tbody>
</table>

### Table 3  
Distribution of polyfungal populations CAC and OCCA in vaginal samples.

<table>
<thead>
<tr>
<th>Species (No. of cases)</th>
<th>Growth on CAC or OCCA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAC</td>
</tr>
<tr>
<td>\textit{Candida albicans}+\textit{C. glabrata} (10)</td>
<td>4</td>
</tr>
<tr>
<td>\textit{Candida albicans}+\textit{C. krusei} (4)</td>
<td>4</td>
</tr>
<tr>
<td>\textit{Candida albicans}+\textit{C. tropicalis} (3)</td>
<td>3</td>
</tr>
<tr>
<td>\textit{Candida albicans}+\textit{C. famata} (3)</td>
<td>2</td>
</tr>
<tr>
<td>\textit{Candida albicans}+\textit{C. kefyr} (1)</td>
<td>1</td>
</tr>
<tr>
<td>Total (21)</td>
<td>14</td>
</tr>
</tbody>
</table>
light blue-green color with a pale border, and were identifiable on CAC. However, in this present study, all non-\textit{C. albicans} Candida species, including \textit{C. rugosa}, produced either white or lavender colonies on CAC, which were not distinguishable from \textit{C. tropicalis} (Table 1). Some authors also reported that the dark bluish-green color might distinguish \textit{C. dubliniensis} from \textit{C. albicans} [21,24,25]. Furthermore, \textit{C. dubliniensis} developed a dark bluish-green color on OCCA [14]. In contrast to the observation of Ghelardi \textit{et al.} [11], our one \textit{C. guilliermondii} strain produced a beige, not blackberry wine, color on OCCA.

We encountered only two investigations dealing with the efficacy of OCCA in the literature. First, Baixench \textit{et al.} [6] used CAC as a reference medium for comparison with OCCA, and observed no difference between CAC and OCCA regarding the presumptive identification of \textit{Candida} spp. A sensitivity of 96.6% and a specificity of 100% for the identification of \textit{C. albicans} at 48 h by OCCA were reported. The authors noted that all of the five \textit{C. tropicalis} and one \textit{C. krusei} were correctly identified on OCCA. However, 45 \textit{C. glabrata} produced variously colored colonies on this medium, i.e., 37.8% maroon, 31.1% beige, 28.9% yellow, and 2.2% violet. More recently, Ghelardi \textit{et al.} [11] reported that distinctive colonies were produced by \textit{C. albicans}, \textit{C. tropicalis}, \textit{C. krusei}, \textit{C. guilliermondii}, \textit{Saccharomyces cerevisiae}, \textit{Trichosporon mucoides}, and \textit{Geotrichum capitatum}. The authors also noted that the sensitivity and specificity of the medium exceeded 99.4% for each of the species. In this investigation, the efficacy of OCCA for \textit{C. albicans} was 95.6% at 72 h (Table 2).

Polyfungal populations are common in clinical samples [7]. However, polyfungal populations may not be detected by the routinely used SCA in practice of mycology. When non-\textit{C. albicans} Candida azole-resistant strains are missed, prompt and appropriate antifungal therapy may be delayed [17]. The frequency of polyfungal populations from stool or rectal swab samples was reported as 7.8% [24], from various clinical samples as 13.3% [6], from clinical samples with undeclared origin as 16.5% [1], and from vaginal samples as 1.3% [20], 2.5% [17], and 4.8% [5]. Ghelardi \textit{et al.} [11] prepared 47 polyfungal suspensions for discrimination on OCCA, and found that this medium allowed the identification and discrimination of yeast mixtures in 42 (89.4%) samples.

In the present study, OCCA is more efficient than CAC (95.2% vs. 66.7%) in detecting polyfungal populations from vaginal samples ($P<0.05$) (Table 3). Moreover, we detect a higher frequency of polyfungal population (13%) in vaginal samples. Our results vary depending on the origin of the samples, and with the choice of chromogenic media such as OCCA, which better detects polyfungal populations. We observed that polyfungal populations are always accompanied by \textit{C. albicans}. The most common combination is \textit{C. albicans}+\textit{C. glabrata} detected in 47.6% of the polyfungal populations. This is a higher frequency than previously reported, at 30% and 46.5% [17,24]. However, Richter \textit{et al.} [3] observed \textit{C. albicans}+\textit{C. glabrata} in 77.7% of polyfungal populations from vaginal samples. In the literature, \textit{C. albicans} is reported to be present in most isolates, a fact explained by the higher incidence of this species in various clinical samples [14].

In conclusion, the performance of two chromogenic media, CAC and OCCA, have several advantages compared to SCA for the recovery and identification of vaginal yeast isolates. Moreover, these media allow easy recognition of polyfungal populations. Both chromogenic media showed 087.5% of sensitivity and specificity for \textit{C. albicans} at 72 h (Table 2). This capability may save the costs of additional conventional diagnostic tests such as GTT, microscopic morphology on CTA, and the API 20C AUX test [17,18]. For \textit{C. albicans} isolates, using the appearance of colony morphology on CAC with either GTT [17] or CTA [23], in comparison with the results of API yeast identification kits, was reported as less expensive.

On the other hand, such strains of \textit{C. albicans} and non-\textit{C. albicans} Candida, sometimes did not only fail to produce the expected colors, but sometimes the produced colors were also incorrect. In particular, most strains of \textit{C. tropicalis} and \textit{C. krusei} were identifiable on CAC, while OCCA failed to show them. However, the rest of the yeast species were not identifiable on either CAC or OCCA. The importance of a laboratory assay that may require up to 72 h for identification, especially presumptive, and may be limited to the identifications of three species (\textit{C. albicans}, \textit{C. tropicalis}, and \textit{C. krusei}) is of questionable value. If the chromogenic medium was used without subsequent biochemical identification, several common species would have been misidentified. Hence, we conclude that chromogenic agars could be integrated as a primary isolation medium into the laboratory workflow.

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