Use of chromogenic medium in the isolation of yeasts from clinical specimens

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Over a 1 year period 3296 specimens submitted for fungal culture were plated onto routine mycological media (RM) and CHROMagar Candida (CaC) to evaluate the capability of CaC to improve on RM. With RM, cultures producing single yeast isolates were identified from 802 specimens. CaC produced similar results, with 76% agreement. Of 761 specimens that yielded a single Candida species by RM, 615 (81%) produced one or more yeast isolates using CaC. Of concern, 132 negative CaC cultures corresponded to specimens that yielded C. albicans alone on RM. When yeasts were recovered, CaC correctly identified 98% of C. albicans, 93% of Candida tropicalis, 96% of Candida glabrata and 100% of Candida krusei based on typical colours. CaC did potentially improve on RM by detecting yeasts in 91 specimens that yielded none by routine methods. CaC was noted to recover more yeast isolates than RM when mixed cultures were detected. Overall, the role of CaC in improving RM appears limited.

INTRODUCTION

Yeasts are now the fourth most common organisms recovered from blood culture in hospitals (Edmond et al., 1999; Rangel-Frausto et al., 1999). In the past, Candida albicans was the aetiological agent in approximately 80% of nosocomial yeast infections (Beck-Sague & Jarvis, 1993). 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 (Rangel-Frausto et al., 1999). This transition has had a 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.

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); 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. Some unusual yeasts may require unique morphological and biochemical studies for identification, occasionally requiring up to 21 days of incubation.

In order to facilitate rapid identification, alternative techniques to standard media such as chromogenic media have been developed. These special media yield microbial colonies with varying pigmentation secondary to chromogenic substrates that react with enzymes secreted by the microorganisms. CHROMagar Candida (CaC) (www.chromagar.com) employs this methodology to allow the differentiation of several candidal yeasts by colour and morphology; it identifies C. albicans by growth as green colonies, C. tropicalis by growth as steel blue colonies and C. krusei as rough, matted, rose-coloured colonies. Other species such as C. glabrata and Candida dubliniensis may also be reliably identified (Hospenthal et al., 2002; Kirkpatrick et al., 1998; Odds & Bernaerts, 1994; Odds & Davidson, 2000; Pfaller et al., 1996). Another potential advantage of chromogenic media is the straightforward identification of mixed yeast infections, which can have a significant clinical bearing (Hospenthal et al., 2002; Lopez-Ribot et al., 1999; Redding et al., 1999; Willinger et al., 2001; Willinger & Manafi, 1999).

Studies assessing the utility of chromogenic media have been performed, but a long-term, large-scale evaluation using one of these media routinely for all samples from a clinical mycology laboratory has not been previously undertaken. We studied the use of CaC in improving routine mycological media (RM) for all specimens referred to the clinical mycology laboratory.
METHODS

Specimens. We prospectively studied all clinical specimens and cultures referred to the clinical mycology laboratory (CML) at Brooke Army Medical Center (BAMC), Fort Sam Houston, Texas, between January 2002 and January 2003. BAMC is a tertiary-care medical facility that does not perform bone marrow or solid organ transplants. The CML assesses clinical samples that are direct referrals, referrals from the bacteria section of the clinical microbiology laboratory and outside referrals from smaller, regional hospitals. Referrals from the bacteria section include fungal colonies on solid media and liquid media from Gram-stain-positive blood culture bottles.

Cultures. The media used routinely (RM) in the CML for primary isolation of fungi differed for typically sterile and non-sterile site specimens. Referred fungi growing on or in bacterial media were plated directly as sterile site specimens. Sterile site specimens were plated onto brain heart infusion medium with 10 % sheep blood, chloramphenicol and gentamicin (BHI) (Becton Dickinson) and potato flake agar (Becton Dickinson). Non-sterile site specimens were plated onto Mycosel agar (Sabouraud dextrose agar containing chloramphenicol and cycloheximide) (Becton Dickinson) and BHI.

Identification of yeast and yeast-like organisms was done by germ-tube testing, observation of morphology on corn meal agar (Becton Dickin-son), Vitek Yeast Biochemical Cards (bioMerieux), and fermentation of glucose, sucrose and cellobiose. Further studies used to identify unusual yeast included API 20C (bioMerieux), growth at 37 °C and cycloheximide) (Becton Dickinson) and BHI.

Among these specimens, when yeasts were recovered on CaC, including 132 (23 %) of 564 isolates recovered by RM did not grow candidal yeasts at all based on expected colour and morphology. Of the remaining five species not typically identifiable on CaC (Candida parapsilosis, Candida guilliermondii, Candida famata, Candida lus-titiae and Candida lipolytica), colony colours were noted, as described previously, in varying shades of white, pink and lavender. Unfortunately, 146 of the 761 single Candida isolates recovered by RM did not grow candidal yeasts at all on CaC, including 132 (23 %) of 564 C. albicans isolates.

The performance of CaC with sterile site specimens was generally better than that seen with non-sterile site specimens, especially dermatological samples (scalp/hair, skin and nail) (Table 2). Agreement was seen between CaC and RM results in 88 % of blood (121/137), 78 % of catheter tip (14/18) and 99 % of cerebrospinal fluid (CSF) (357/359) cultures. Discordance seen with sterile site specimens (blood, catheter tips ans CSF) was most commonly due to lack of any growth on CaC. For blood cultures, 13 samples were negative by CaC but positive for a Candida species by RM. One CaC culture recovered a green yeast colony that corresponded to a RM culture that was negative. The patient from whom this blood sample was obtained had no other positive fungal cultures during their hospitalization and thus the significance of this result is unknown. Two CaC blood specimens each grew two differing colony types that did not correlate with the matched single isolates recovered by RM. For one of these patients, a second blood sample and catheter tip removed the same day samples consisted of specimens from other sites including the oral cavity, genital tract, soft tissues, bone and those listed as ‘unknown’ or ‘other’.

Culture results

RM identified a single yeast isolate from 802 (24 %) of the specimens and more than one yeast from 51 (2 %) of the specimens. No yeasts were recovered from 2443 (74 %) of the specimens. When compared only by the number of yeasts recovered on each plate (not matching colour or colony characteristics), CaC produced similar results from 2983 of the tested specimens (91 % agreement). Agreement was highest for specimens without growth on RM (96 %, 2352/2443), with lower agreement when RM recovered a single yeast (76 %, 606/802) or multiple yeasts (49 %, 25/51). CaC recovered yeasts from a total of 91 specimens that yielded no growth by RM, but failed to detect yeast growth from 164 of 853 (19 %) specimens that did produce yeast growth using RM. Of the mixed cultures, RM revealed more than one yeast isolate from 51 specimens, whereas CaC recovered more than one yeast isolate from 83 specimens. In total, multiple isolates were recovered from RM, CaC or both for 104 patient specimens. Agreement between the methods was seen with 23 (22 %) of these, with RM recovering more isolates in 26 and CaC recovering more isolates in 55.

RESULTS

Specimens

From January 2002 to January 2003, 3554 clinical specimens were evaluated by the CML. Of these samples, 258 were duplicates or rejected for other reasons. Consequently 3296 specimens were plated onto media and had results available for analysis. Specimens from sterile sites included 359 cerebrospinal fluid, 137 blood and 18 catheter tip samples. Predominately non-sterile sites from which samples were submitted included respiratory (467), urine (204), nail (720), scalp/hair (221) and skin (319). Respiratory samples included specimens labelled sputum, bronchialveolar lavage, tracheal aspirate, pleural fluid and lung. The remaining 851
produced two different yeasts on both RM and CaC, supporting the CaC results. Discordance between catheter tip cultures resulted from the recovery of no yeasts by CaC from three specimens and the recovery of only one of two isolates from another sample. CSF specimens produced discordant yeast growth on CaC in two cases (out of 359 total submitted specimens), in each of which a review of the clinical data found that the most likely cause was culture or specimen contamination.

### Table 1. Morphology and recovery of Candida by CHROMagar Candida compared with routine media

Data are presented for all RM cultures that yielded only one Candida species.

<table>
<thead>
<tr>
<th>Candida species</th>
<th>No. of species recovered by RM</th>
<th>CHROMagar Candida result</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Colour/morphology</td>
</tr>
<tr>
<td></td>
<td></td>
<td>as expected (no.)</td>
</tr>
<tr>
<td>C. albicans</td>
<td>564</td>
<td>422</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>71</td>
<td>64</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>63</td>
<td>–‡</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>43</td>
<td>40</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>11</td>
<td>–‡</td>
</tr>
<tr>
<td>C. famata</td>
<td>3</td>
<td>–‡</td>
</tr>
<tr>
<td>C. krusei</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>3</td>
<td>–‡</td>
</tr>
<tr>
<td>C. lipolytica</td>
<td>1</td>
<td>–‡</td>
</tr>
</tbody>
</table>

*Four lavender, three tan/ivory, two pink and one blue isolates.
†Two pink and one green isolates.
‡No identifying colour is claimed by the manufacturer or other research.
§Three lavender isolates.

### Table 2. Performance of CHROMagar Candida by site of specimen collection

<table>
<thead>
<tr>
<th>Site of collection</th>
<th>No. of specimens</th>
<th>No. of specimens with agreement between RM and CaC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>137</td>
<td>121 (88)</td>
</tr>
<tr>
<td>Catheter tip</td>
<td>18</td>
<td>14 (78)</td>
</tr>
<tr>
<td>CSF</td>
<td>359</td>
<td>357 (99)</td>
</tr>
<tr>
<td>Genital</td>
<td>73</td>
<td>62 (85)</td>
</tr>
<tr>
<td>Nail</td>
<td>720</td>
<td>660 (92)</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>467</td>
<td>364 (78)</td>
</tr>
<tr>
<td>Respiratory</td>
<td>22</td>
<td>20 (91)</td>
</tr>
<tr>
<td>Scalp/hair</td>
<td>221</td>
<td>215 (97)</td>
</tr>
<tr>
<td>Skin</td>
<td>319</td>
<td>293 (92)</td>
</tr>
<tr>
<td>Urine</td>
<td>204</td>
<td>166 (81)</td>
</tr>
</tbody>
</table>

*Agreement is based on recovery on CaC of the same number of yeast species compared with results obtained on RM. Values in parentheses indicate the percentage of specimens in agreement.

### DISCUSSION

Previous evaluation of chromogenic media in the recovery of fungi directly from clinical specimens has been limited by small sample sizes, inclusion of only selected clinical samples or lack of comparison to routine media. We prospectively evaluated the utility of chromogenic media to improve on media routinely used by our hospital mycology laboratory in all clinical specimens submitted during a 12 month period. We expected the most promising application of chromogenic media in this clinical setting to be the ability to identify mixed infections and to improve the detection of yeast. However, CaC did not clearly enhance traditional techniques of fungal identification.

Our study examined the culture results of 3296 clinical isolates, 853 of which produced yeast growth on RM. The largest study reported in the literature examined 6150 clinical specimens (Bouchara et al., 1996). Most of these specimens were non-sterile site surveillance samples and only 366 were also plated on Sabouraud medium for comparison. The three largest studies to compare CaC to a routine medium, Sabouraud medium either with or without chloramphenicol, examined 951–1784 specimens. These included 1784 vaginal swabs in the largest comparative study (Houang et al., 1997), 951 mostly genital and stool specimens in another (Baumgartner et al., 1996) and 1150 unspecified clinical samples in the third (Willinger & Manafi, 1999). Smaller comparative studies have also been reported (Odds & Bernaerts, 1994; Pfaller et al., 1996; Willinger et al., 2001; Ainscough & Kibbler, 1998; Bernal et al., 1996; Freydiere et al., 1997; Momani, 2000), most of which examined non-sterile site specimens, commonly surveillance cultures. None of these
other studies performed long-term parallel comparative examination of CaC versus RM with all samples submitted to their hospital mycology laboratories.

In this study the chromogenic medium CHROMagar Candida failed to detect yeast growth from 19% of specimens that did produce yeast growth using routine laboratory culture, including 132 specimen cultures that produced *C. albicans* on RM. In the other studies referenced above, CaC generally recovered at least 90% of those yeasts recovered by RM, and often more yeasts were recovered by CaC than RM. However, as also stated previously, the clinical specimens examined in these other studies generally were from surveillance sites. The sites included were those that typically yield high concentrations of yeasts.

CaC did potentially improve on RM by detecting yeasts in 91 specimens that produced no yeast growth by routine methods and detecting more yeasts than RM in 38 additional specimens. Chromogenic media have been reported to enhance the identification of mixed cultures to varying degrees over traditional media (Pfaller et al., 1996; Willinger & Manafi, 1999; Baumgartner et al., 1996; Beighton et al., 1995).

Overall, 97% (464/477) of the *C. albicans*, *C. tropicalis* and *C. krusei* isolates recovered were noted to produce the colours and morphology described by the manufacturer of CaC. We also described alternative colours of some yeasts on CaC that are not consistent with the traditionally identifiable colours. This may represent recovery of yeasts of different species on some plates or colour variability among strains of these species, as has been reported by others (Cooke et al., 2002). This reveals a limitation of our study in that we did not identify to the species level all yeasts detected on CaC in parallel with RM.

Chromogenic agar carries the potential of improving identification of yeast, especially in mixed cultures. Our evaluation of CaC as a routine fungal medium revealed disappointing results. Its role as a primary medium to improve the recovery of yeasts or the detection of mixed cultures appears limited.

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