Direct Isolation of Candida spp. from Blood Cultures on the Chromogenic Medium CHROMagar Candida

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CHROMagar Candida is a selective and differential chromogenic medium that has been shown to be useful for identification of Candida albicans, Candida krusei, Candida tropicalis, and perhaps Candida glabrata. Colony morphology and color have been well defined when CHROMagar Candida has been used to isolate yeast directly from clinical specimens, including stool, urine, respiratory, vaginal, oropharyngeal, and esophageal sources. Direct isolation of yeast on CHROMagar Candida from blood cultures has not been evaluated. We evaluated whether the color and colony characteristics produced by Candida spp. on CHROMagar Candida were altered when yeasts were isolated directly from blood cultures. Fifty clinical isolates of Candida were inoculated into aerobic and anaerobic blood culture bottles and incubated at 35°C in an automated blood culture system. When growth was detected, an aliquot was removed and plated onto CHROMagar Candida. As a control, CHROMagar Candida plates were inoculated with the same isolate of yeast grown on Sabouraud dextrose agar simultaneously. No significant difference was detected in color or colony morphology between the blood and control isolates in any of the tested organisms. All C. albicans (n = 12), C. tropicalis (n = 12), C. glabrata (n = 9), and C. krusei (n = 5) isolates exhibited the expected species-specific colony characteristics and color, whether isolated directly from blood or from control cultures. CHROMagar Candida can be reliably used for direct isolation of yeast from blood cultures. Direct isolation could allow mycology laboratories to more rapidly identify Candida spp., enable clinicians to more quickly make antifungal agent selections, and potentially decrease patient morbidity and mortality.

Candidiasis is an increasingly common problem in hospitalized patients, with epidemiologic surveys revealing that Candida spp. are now the fourth most common pathogens isolated from the blood of hospitalized patients (7, 21). Candida albicans historically has been the predominant cause of candidiasis. In the 1980s C. albicans accounted for greater than 80% of all candidal isolates recovered from nosocomial yeast infections (4). More recently, non-albicans Candida (NAC) species have been recovered with increasing frequency. C. albicans now accounts for less than 50% of all candidal blood isolates in a recent review (21). Increasingly, Candida glabrata, Candida parapsilosis, Candida tropicalis, and other NAC are the yeast species responsible for candidiasis. Importantly, many NAC have decreased susceptibility to antifungal agents. Specifically, Candida krusei and many C. glabrata demonstrate decreased susceptibility to fluconazole. Clinicians now depend on identification of Candida to the species level in order to optimize the selection of antifungal agents and to allow them to provide the best possible patient care.

Several brands of chromogenic media have been developed to produce rapid yeast identification. These media contain chromogenic substrates that react with enzymes secreted by microorganisms producing colonies with various pigmentation. These enzymes are specific, allowing organisms to be identified to the species level by their color and colony characteristics. CHROMagar Candida has been shown to allow differentiation of candidal yeasts by color and morphology (18). The manufacturer currently advertises its product as able to detect and differentiate three species of Candida. Per the package insert for CHROMagar Candida, the product identifies C. albicans by growth as light to medium green colonies, C. tropicalis by growth as steel blue colonies accompanied by purple pigment diffusion into surrounding agar, and C. krusei by growth as large, fuzzy, rose-colored colonies with white edges, all after incubation for 48 h at 37°C. This media has been demonstrated to identify C. albicans, C. krusei, and C. tropicalis in several studies (3, 6–11, 18, 19). Recent reports have suggested that the dark green appearance of Candida dubliniensis can also be reliably distinguished from that of C. albicans (12, 13, 15, 19). The identification of C. glabrata remains controversial. Several authors maintain that C. glabrata is readily identifiable on CHROMagar Candida with its characteristic dark pink to purple coloration (6, 9, 11, 20). The colonies are sometimes accompanied by a diffusion of pigment into the surrounding agar (9). Other authors consider the media unreliable for the identification of C. glabrata (5, 10, 8, 14, 18, 19).

Routine use of chromogenic media carries the potential for cost savings in the clinical microbiology laboratory (1, 14). Use of these media could potentially save the time and expense of performing assimilation panels and other fermentative or biochemical testing. In addition, use of CHROMagar Candida can also improve the ability of the mycology laboratory to rapidly identify mixed yeast infections (9, 15, 18).

Small studies have been designed to test strategies employing CHROMagar Candida, although most of these early studies evaluated stock isolates rather than clinical specimens (15, 18). Other studies have evaluated CHROMagar Candida as a primary medium to identify yeasts directly from clinical spec-
imems, including stool, urine, respiratory, vaginal, oropharyngeal, and esophageal sources (1–3, 5, 8, 10, 12, 13, 15, 22). To our knowledge, the isolation of Candida spp. directly from blood cultures onto CHROMagar Candida has not been studied. Pigments in blood components and broth-based blood culture media could potentially alter the distinct color generated by yeast on CHROMagar Candida. Therefore, this study was designed to determine whether the color generated by yeast on CHROMagar Candida was altered when isolated directly from blood cultures.

MATERIALS AND METHODS

Fifty strains of Candida originally isolated from clinical specimens submitted to the Brooke Army Medical Center mycology laboratory were used. These included isolates of C. albicans (n = 12), C. tropicalis (n = 12), C. glabrata (n = 9), C. krusei (n = 5), Candida lusitaniae (n = 3), C. parapsilosis (n = 3), Candida guilliermondii (n = 2), Candida kefyr (n = 2), Candida famata (n = 1), and Candida rugosa (n = 1). Each specimen was randomly assigned a number from 1 to 50, and investigators were blinded to the identities until the study was completed. Frozen yeast isolates were subcultured twice onto Sabouraud dextrose agar (SDA) (BBL Media, Sparks, Md.) to ensure isolation of pure colonies. After 48 h of incubation, a sample of each specimen was suspended in 5 ml of sterile normal saline and adjusted by transmittance at 530 nm (Spectronic 20D; Milton Roy, Berlin, Germany) to a 0.5 McFarland standard (Becton Dickinson and Company, Sparks, Md.). The resulting suspension predictably should contain approximately 1 × 108 to 5 × 108 yeast cells/ml. A 1:100 dilution of each yeast suspension was then performed to produce a final concentration of 1 × 106 to 5 × 106 yeast cells/ml. A volume of 0.1 ml of each isolate was introduced into each set of blood cultures, one aerobic bottle and one anaerobic bottle, to produce a final inoculum of approximately 1 × 105 to 5 × 105 yeast cells. Concentrations were verified by plating onto SDA and performing counts of serial dilutions.

The blood culture bottles utilized in this study were the BD BACTEC PLUS Aerobic/F and Anaerobic/F (Becton Dickinson and Company). All bottles were inoculated with 10 ml of fresh, whole blood from healthy volunteers. The study was approved by the Brooke Army Medical Center institutional review board. After the addition of yeast cells, inoculated bottles were immediately placed in the automated BACTEC 9240 incubator system. This system incubates specimens at 35°C with continuous agitation and uses a fluorescent technology to detect the quantity and rate of CO2 production (indicative of microbial growth), every 10 min. Blood culture bottles were removed from the incubator after the automated system determined that they were positive. A 0.1-ml aliquot was withdrawn from each positive bottle and plated (four quadrant streaking) onto CHROMagar Candida (CHROMagar Microbiology, Paris, France) medium. A control specimen was plated in parallel directly from SDA to CHROMagar Candida. The specimens were then incubated at 30°C and observed and photographed on days 1, 2, 3, 4, and 7. The incubation temperature of 30°C was selected, as this temperature has been used successfully in prior studies with this chromogenic medium. The 11 isolates tested in this study among this group included C. lusitaniae (n = 3), C. parapsilosis (n = 3), C. guilliermondii (n = 2), C. kefyr (n = 2), and C. famata (n = 1). There were no significant differences in the colors produced on CHROMagar Candida by any of these species whether isolated from blood or a control specimen. As previously reported (9), we again noted a distinct appearance produced by C. rugosa on CHROMagar Candida. Candida rugosa grew in small, dry colonies of a brilliant blue color that possessed a pale or white border.

RESULTS

The color and morphology characteristics of all fifty isolates on CHROMagar Candida were directly compared from blood and control sources. Yeast growing directly over the first quadrant of streaking (where the higher quantity of blood left a red stain) demonstrated a slightly darker hue compared to that seen in the other three quadrants or on the control plates (Fig. 1). This color variation was slight and most apparent after 24 h of incubation, waning over the following 7 days. Color variation was minimally apparent at 48 h, the time at which CHROMagar Microbiology recommends reading the plates. This was a consistent finding with all 50 isolates.

Thirty-eight isolates of the four Candida spp. known to generate a distinct, identifiable color on CHROMagar Candida were utilized in this study, including C. albicans (n = 12), C. tropicalis (n = 12), C. glabrata (n = 9), and C. krusei (n = 5). No significant difference was noted between the color produced from blood or control specimens. The expected color used to identify each of these species also did not appear to be altered by direct isolation from blood. All 24 of the C. albicans and C. tropicalis isolates were readily identifiable by 48 h. All five C. krusei isolates produced the distinctive large, dry, pink colonies by 24 h. C. glabrata isolates form small colonies that are reported to produce a pink to dark purple coloration, sometimes with diffusion of pigment into the surrounding agar. None of our nine C. glabrata isolates demonstrated these characteristics after 48 h of incubation, although eight isolates did do so by 72 h. The ninth C. glabrata isolate did not develop this dark purple color until 96 h and did not have any pigment diffusion into the surrounding agar.

Other Candida species typically produce small, creamy colonies that are white, pale pink, or light lavender on CHROMagar Candida and are not distinguishable to the species level with this chromogenic medium. The 11 isolates tested in this study among this group included C. lusitaniae (n = 3), C. parapsilosis (n = 3), C. guilliermondii (n = 2), C. kefyr (n = 2), and C. famata (n = 1). There were no significant differences in the colors produced on CHROMagar Candida by any of these species whether isolated from blood or a control specimen. As previously reported (9), we again noted a distinct appearance produced by C. rugosa on CHROMagar Candida. Candida rugosa grew in small, dry colonies of a brilliant blue color that possessed a pale or white border.

DISCUSSION

The four major Candida spp. that have been reported to be identifiable on CHROMagar Candida, C. albicans, C. glabrata, C. krusei, and C. tropicalis, were readily identifiable when isolated directly from blood in this study. Yeast growing directly over the blood quadrant produced a slightly darker hue, but this ultimately did not interfere with the ability to correctly identify each Candida spp. Our initial concern that the color produced on CHROMagar Candida might be altered by the presence of heme pigment from blood and therefore impede our ability to recognize the characteristic colors of C. albicans, C. krusei, C. tropicalis, and C. glabrata was not realized. Overall, there were no significant differences noted in color or colony morphology on any of our isolates whether plated from blood or control SDA plates.

NAC isolates, including C. lusitaniae, C. parapsilosis, C. guilliermondii, C. kefyr, and C. famata, also produced no difference in color or colony morphology when plated from blood or SDA onto CHROMagar Candida. As has been previously reported, these species were not readily distinguishable from one another on this chromogenic medium. Conversely, the unique color and colony morphology of C. rugosa may allow the identification of this species on CHROMagar Candida. Candida rugosa is uncommonly isolated from clinical specimens, and a study including more isolates is required to confirm this finding.

CHROMagar Candida can be reliably used for primary isolation of yeast from clinical specimens, including blood cul-

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FIG. 1. Growth of *C. albicans* (A and B), *C. krusei* (C and D), *C. tropicalis* (E and F), and *C. glabrata* (G and H) on CHROMagar Candida, with direct isolation from blood culture bottled medium (A, C, E, and G) compared to that seen with inoculation from culture on standard solid medium (B, D, F, and H).
which could allow mycology laboratories to more rapidly identify clinically important Candida spp. While potentially decreasing laboratory costs (1, 14), this capability will also enable clinicians to more rapidly make appropriate antifungal choices, decreasing patient morbidity and mortality.

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REFERENCES