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Application of CHROMagar Candida for Rapid Screening of Clinical Specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida* (*Torulopsis*) *glabrata*

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CHROMagar Candida is a new differential culture medium that allows selective isolation of yeasts and simultaneously identifies colonies of *Candida albicans*, *C. tropicalis*, and *C. krusei*. We evaluated the use of this medium with 316 yeast isolates including 247 isolated directly on CHROMagar from clinical material. Over 95% of stock and clinical isolates of *C. albicans*, *C. tropicalis*, and *C. krusei* were correctly identified on the basis of colony morphology and pigmentation on CHROMagar. Additionally, CHROMagar also allowed the identification of *C.* (*Torulopsis*) *glabrata* at a similar level of accuracy. The overall agreement between two observers in reading the CHROMagar plates was 95%. Growth of *Candida* sp. isolates on CHROMagar had no adverse effect on antifungal MICs or Vitek identification results. In parallel, cultures of 548 stool and rectal swab specimens set up on CHROMagar and Sabouraud glucose agar (SGA) were positive in 234 instances. CHROMagar was positive and SGA was negative for 11 specimens, and CHROMagar was negative and SGA was positive for 18 specimens. A single yeast species was isolated on both media from 162 specimens, and in 146 (90%) of these specimens the same species was detected on both CHROMagar and SGA. A total of 43 of the 234 positive cultures contained mixtures of yeast species. Twenty (47%) of these mixed cultures were detected only on CHROMagar. CHROMagar is extremely useful in making a rapid presumptive identification of common yeast species. This capability plus the ability to detect mixed cultures of *Candida* spp. promises to improve and streamline the work flow in the mycology and clinical microbiology laboratory.

Although the pathogenesis of hematogenously disseminated candidiasis is not completely understood, it is thought that the first step in the development of this infectious process is the colonization of the gastrointestinal tract or oropharynx by *Candida* spp. (4, 5). Surveillance cultures of selected body sites are a method of evaluating the colonization by *Candida* species of patients at high risk of infection (1–3, 6, 12). Several studies have documented the link between colonization and infection, and this appears to hold for *Candida albicans* and several other *Candida* species including *C. tropicalis*, *C. krusei*, and *C.* (*Torulopsis*) *glabrata* (4–6, 10, 12, 13).

The emergence of *Candida* species other than *C. albicans* as important agents of infection is a concern in several major institutions (4–6, 9, 11, 12, 15). Although the reasons for the emergence of these agents are not completely known, one important factor may be the relative lack of susceptibility to fluconazole and other azoles (4, 5, 9, 11, 15). The three species noted above [*C. tropicalis*, *C. krusei*, and *C.* (*Torulopsis*) *glabrata*] constitute the majority of species other than *C. albicans* isolated in most institutions and have been observed to be 4- to 32-fold less susceptible to fluconazole than *C. albicans* (9, 11). Given the potential for selection of these less susceptible species, ongoing surveillance may be prudent (5). Clinical laboratories may need to expand their yeast identification capabilities in order to facilitate these surveillance efforts (5).

Ideally, laboratories should be able to simultaneously detect and identify *C. albicans* and the major *Candida* species other than *C. albicans* in clinical specimens. A recently developed agar medium, CHROMagar Candida, appears to meet these criteria (1–3). CHROMagar is a selective and differential medium that allows selective isolation of yeasts and simultaneously identifies (by color reactions and colony morphology) colonies of *C. albicans*, *C. tropicalis*, and *C. krusei* with a high degree of accuracy (3). It facilitates the detection and identification of yeasts from mixed cultures and can provide results 24 to 48 h sooner than standard isolation and identification procedures (1–3). Clearly, given the concerns of emergence of species less susceptible to fluconazole, a rapid screening approach using CHROMagar may aid in optimal utilization of this and other antifungal agents.

In the present study, we sought to evaluate the usefulness of CHROMagar for detection and presumptive identification of yeast species in surveillance cultures obtained from patients hospitalized in our surgical and neonatal intensive care units (ICUs). Our objectives were (i) to evaluate the accuracy of CHROMagar for the identification of *Candida* spp. from stock cultures and from clinical specimens, (ii) to evaluate the effect (if any) of CHROMagar on standard identification and susceptibility testing results, and (iii) to evaluate the interobserver variation in reading and interpreting the results of CHROMagar cultures.

MATERIALS AND METHODS

Culture media. CHROMagar Candida was purchased as powdered medium from the CHROMagar Company, Paris, France. In addition to peptone (10 g/liter), glucose (20 g/liter), and agar (15 g/liter), the medium contained chloramphenicol (0.5 g/liter) and chromogenic mix (2 g/liter). The medium was prepared according to the manufacturer's instructions and dispensed into petri dishes (20 ml into 100-mm-diameter dishes). Sabouraud glucose agar (SGA) containing chloramphenicol (0.05 g/liter) and gentamicin (0.04 g/liter) and potato dextrose agar (PDA) were purchased from Remel (Lenexa, Kans.).

Organisms. A total of 69 isolates of *Candida* species from stock cultures were used to assess the accuracy of CHROMagar identification and to determine the effect of CHROMagar on standard identification and antifungal susceptibility testing results. All isolates were inoculated in parallel onto CHROMagar and PDA. The isolates were all from clinical sources and included *C. albicans* (14

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isolates), *C. tropicalis* (13 isolates), *C. krusei* (8 isolates), *C.* (*Torulopsis*) *glabrata* (10 isolates), and 24 isolates of other *Candida* species (11 *C. parapsilosis*, 11 *C. lusitaniae*, and 2 *C. lipolytica*). All isolates were identified by standard methods (14) and were stored as water suspensions at ambient temperature.

Clinical specimens. A total of 547 clinical specimens of stool or rectal swabs obtained from patients hospitalized in either the surgical ICU or the neonatal ICU were submitted for yeast surveillance cultures. All specimens were inoculated in parallel onto CHROMagar and SGA. Following inoculation, the culture plates were incubated in air at 30°C and were inspected daily for a total of 7 days.

Identification methods. All yeast isolates observed on CHROMagar were identified by colony morphology and pigmentation according to the manufacturer's instructions and as described by Odds and Bernaerts (3). Isolates from SGA and PDA, as well as CHROMagar plates, were also identified by the Vitek yeast identification system (bio Merieux Vitek, Hazelwood, Mo.) (8) supplemented by standard methods and morphology as necessary (14).

Antifungal susceptibility testing. Antifungal susceptibility testing was performed by using the standard methods of the National Committee for Clinical Laboratory Standards (Villanova, Pa.) as described previously (7). The 69 stock isolates of *Candida* spp. were grown on both CHROMagar and PDA and then tested for susceptibility to amphotericin B, flucytosine, fluconazole, itraconazole, and the experimental triazole D0870 (Zeneca, Cheshire, England). The MICs for each drug-organism combination were compared for inocula prepared from the two different media.

Study design. In all phases of the study, organisms were grown on both CHROMagar and either PDA (stock isolates) or SGA (clinical isolates). In the first phase of the study, stock cultures of *Candida* spp. were used to assess the accuracy of CHROMagar identification (objective 1) and the effect of CHROMagar on standard identification and antifungal susceptibility tests (objective 2). Stock cultures were plated onto both CHROMagar and PDA. After 48 h of incubation, the CHROMagar plates were read by a technologist blinded to the identity of the isolates and a species identification was assigned on the basis of previously published criteria (3). The isolates from both CHROMagar and PDA were also identified by the Vitek yeast identification system (bio Merieux Vitek) and tested for susceptibility to a panel of antifungal agents. The comparison of the antifungal susceptibility values and the Vitek identification results for isolates taken from the two media allowed us to assess the effect of CHROMagar on these test procedures. In the second phase of the study, we examined the ability of CHROMagar to detect and identify yeast isolates in clinical specimens. Surveillance stool specimens were plated onto both CHROMagar and SGA. The plates were incubated for 48 h and examined independently by two observers. All colonies on CHROMagar were identified on the basis of morphology and pigmentation (3), and yeast isolates from both CHROMagar and SGA were identified to the species level by the Vitek system. This approach allowed us to address the clinical performance of CHROMagar (objective 1) and the level of agreement among technologists in reading and interpreting cultures on CHROMagar medium (objective 3).

RESULTS AND DISCUSSION

By adhering to the manufacturer's guidelines and the published criteria of Odds and Bernaerts (3), we were able to identify to the species level 100% of the stock isolates of *C. albicans*, *C. tropicalis*, and *C. krusei*. In addition, by using the color photographs supplied by the manufacturer and those presented in the publication of Odds and Bernaerts (3), we were able to identify 9 of 10 stock isolates of *C.* (*Torulopsis*) *glabrata* (dark pink colonies with pale edges) and differentiate them from the other *Candida* species. The remaining 24 isolates of *Candida* spp. other than *C. albicans*, *C. krusei*, *C. tropicalis*, and *C.* (*Torulopsis*) *glabrata* were not distinguishable from one another (white, pale pink, grayish purple) and were lumped together as *Candida* spp. (data not shown).

Growth of stock cultures on CHROMagar had no effect on antifungal MICs or the Vitek identification results. The overall agreement (percent within 1 $\log_2$ dilution) among MICs obtained with inocula prepared from CHROMagar versus PDA was 99% (358 of 360 MIC determinations). Likewise, 92% of Vitek identifications of isolates grown on CHROMagar matched those of isolates grown on PDA.

The clinical performance of CHROMagar in detecting yeast species in surveillance cultures of stool is summarized in Table 1. A total of 548 specimens were cultured on both CHROMagar and SGA. No growth was observed on both CHROMagar and SGA plates in 314 specimens. A total of 234 specimens were positive for one or more yeast species on either CHROMagar (215 specimens) or SGA (222 specimens). CHROMagar was positive and SGA was negative for 11 specimens, and CHROMagar was negative and SGA was positive for 18 specimens. Both CHROMagar and SGA were positive for yeast species in 205 specimens. A single yeast species was isolated on both media from 162 specimens, and for 146 (90%) of these specimens the same species was detected on both CHROMagar and SGA. *C. albicans* accounted for 118 (81%) of the 146 single isolates detected on both media.

TABLE 1. Comparison of CHROMagar and SGA for detection of yeast species in surveillance cultures

Result	No. of cultures	
	1 species	>1 species
CHROMagar negative and SGA negative	314	
CHROMagar positive and/or SGA positive	234	
CHROMagar positive and SGA negative	11	
CHROMagar negative and SGA positive	18	
CHROMagar positive and SGA positive	205	
CHROMagar and SGA same	146	15
CHROMagar and SGA different	16	28

More than one yeast species was detected in 43 (7.8%) of the specimens (Table 1). CHROMagar and SGA detected the same mixture of species in 15 specimens and a different mixture in 28 specimens (Table 2). In 20 of the 43 specimens (47%), CHROMagar detected two or more species when SGA detected only one or none. Conversely, in two of the 43 specimens (5%), SGA detected two species when CHROMagar detected only one. Although CHROMagar and SGA detected different mixtures of yeast isolates in 70% (30 of 43 specimens) of the specimens, at least one of the species from 40 (93%) of the 43 mixed cultures was detected on both media (Table 2).

Overall, 295 yeast isolates were detected in the 234 positive specimens (Table 3). A total of 207 isolates were detected on both media, 49 isolates were detected on CHROMagar only, and 39 isolates were detected on SGA only. CHROMagar appeared somewhat more sensitive than SGA in detecting *C. tropicalis* and less sensitive in detecting *C. parapsilosis*. *C. albicans*, *C.* (*Torulopsis*) *glabrata*, *C. tropicalis*, and *C. krusei* accounted for 87% of the species isolated, with *C. albicans* being the most common (58% of all isolates). CHROMagar correctly identified 94% of *C. glabrata* isolates, 95% of *C. albicans* isolates, and 100% of *C. tropicalis* and *C. krusei* isolates. None of the remaining species were misidentified as one of these four species.

The agreement between the two observers reading the CHROMagar plates was 96% for *C. albicans*, 90% for *C.* (*Torulopsis*) *glabrata*, 66% (two of three) for *C. krusei*, and 100% for *C. tropicalis* and *Candida* spp. Overall agreement between the two observers was 95%.

The results of this study confirm those of Odds and Bernaerts (3) regarding the accuracy of CHROMagar in providing a presumptive identification of *C. albicans*, *C. tropicalis*, and *C. krusei*. Over 95% of stock and clinical isolates of these species were correctly identified on the basis of colony morphology and pigmentation on CHROMagar. Additionally, in our hands CHROMagar also allowed the identification of *C.* (*Torulopsis*) *glabrata* at a similar level of accuracy. The medium was highly selective for yeast isolates. Minimal bacterial contamination was observed despite the fact that we were dealing with surveillance stool specimens. CHROMagar supported the growth of *Candida* spp. to the same degree that we observed with SGA. Although it appeared that CHROMagar may be more

TABLE 2. Detection of multiple yeast species in surveillance cultures using CHROMagar

Species detected with CHROMagar	Species detected with SGA	No. of positive cultures
C. albicans plus *C.* (*Torulopsis*) *glabrata*	None	1
C. albicans plus *C.* (*Torulopsis*) *glabrata*	*C. albicans* plus *C.* (*Torulopsis*) *glabrata*	9
C. albicans plus *C.* (*Torulopsis*) *glabrata*	*C. albicans*	3
C. albicans plus *C.* (*Torulopsis*) *glabrata*	*C. albicans* plus *C. parapsilosis*	1
C. albicans plus *Candida* sp.	*C. albicans*	4
C. albicans plus *Candida* sp.	*C. albicans* plus *C.* (*Torulopsis*) *glabrata*	1
C. albicans plus *C. tropicalis*	*C. albicans*	1
C. albicans plus *C.* (*Torulopsis*) *glabrata* and *C. tropicalis*	*C. albicans* plus *C.* (*Torulopsis*) *glabrata* and *C. tropicalis*	1
C. albicans plus *C.* (*Torulopsis*) *glabrata* and *C. tropicalis*	*C. albicans* plus *C.* (*Torulopsis*) *glabrata*	1
C. (*Torulopsis*) *glabrata* plus *C. albicans*	*C.* (*Torulopsis*) *glabrata*	6
C. (*Torulopsis*) *glabrata* plus *C. tropicalis*	*C.* (*Torulopsis*) *glabrata*	1
C. (*Torulopsis*) *glabrata* plus *C. tropicalis*	*C.* (*Torulopsis*) *glabrata* plus *C. tropicalis*	2
C. (*Torulopsis*) *glabrata* plus *C. tropicalis*	*C.* (*Torulopsis*) *glabrata* plus *C. albicans*	1
C. (*Torulopsis*) *glabrata* plus *C. tropicalis*	*C.* (*Torulopsis*) *glabrata* plus *C. parapsilosis*	1
C. (*Torulopsis*) *glabrata* plus *Candida* sp.	*C.* (*Torulopsis*) *glabrata*	3
C. (*Torulopsis*) *glabrata* plus *C. parapsilosis*	*C.* (*Torulopsis*) *glabrata* plus *C. parapsilosis*	1
C. (*Torulopsis*) *glabrata* plus *Saccharomyces cerevisiae*	*C.* (*Torulopsis*) *glabrata* plus *C. parapsilosis*	1
C. tropicalis plus *C. parapsilosis*	*C. albicans*	1
C. krusei plus *Candida* spp.	*C. krusei* plus *C. parapsilosis*	2
C. krusei	*C. krusei* plus *C. parapsilosis*	1
C. albicans	*C. albicans* plus *C. krusei*	1

sensitive than SGA for detecting *C. tropicalis* and less sensitive for detecting *C. parapsilosis*, there were no obvious differences in growth of these species on the two media, and the reasons for the differences in detection of the two species in clinical specimens are unknown at present. Finally, cultures performed on this medium were relatively easy to read and interpret as demonstrated by the excellent level of agreement between two technologists reading the clinical surveillance cultures independently.

Although CHROMagar appears to be quite accurate in identifying the most common *Candida* species, it is not proposed as a substitute for standard identification protocols (3). Similarly, mere species identification alone may not obviate the need for in vitro assessment of antifungal susceptibility in certain clinical situations. In this regard, it is imperative that isolates can be taken directly from CHROMagar and used in standard identification and susceptibility testing procedures without adverse effects on the accuracy of the results. We have demonstrated that comparable results for both identification and antifungal susceptibility testing may be obtained with inocula taken directly from CHROMagar and PDA. Thus, the use of CHROMagar does not necessitate additional subcultures prior to performing confirmatory or supplemental testing in the clinical laboratory.

TABLE 3. Detection of yeast isolates in surveillance cultures using CHROMagar and SGA[a]

Species (no. of isolates [295])	No. of isolates detected on:		
	Both media [207]	CHROMagar only [49]	SGA only [39]
C. albicans (171)	140	14	17
C. (*Torulopsis*) *glabrata* (59)	44	9	6
C. tropicalis (22)	9	11	2
C. parapsilosis (24)	9	3	12
C. krusei (4)	3		1
C. lusitaniae (1)			1
Candida sp. (12)		12	
Saccharomyces cerevisiae (2)	2		

[a] Totals are given in brackets.

In studies by Odds and Bernaerts (3), Louwagie et al. (1), and Moyer et al. (2), a major advantage of CHROMagar was the ability to detect mixed cultures of yeasts in clinical specimens. In all three previous studies, CHROMagar was superior to other routine and selective media in detecting multiple *Candida* species in both clinical and stock cultures. Our results are in agreement with these prior studies. We found that 43 (18%) of 234 positive cultures contained mixtures of yeast species and that 20 (47%) of these mixed cultures would not have been detected with SGA alone (Table 2). In agreement with the earlier studies (1–3), the most common mixtures observed in the present study were either *C. albicans* plus *C.* (*Torulopsis*) *glabrata* or *C.* (*Torulopsis*) *glabrata* plus *C. tropicalis*. Thus, CHROMagar not only facilitates the detection of mixed cultures but also allows for a presumptive identification to the species level of isolates within the mixture without the need for additional subcultures.

The role of CHROMagar in the clinical microbiology laboratory will vary depending upon the patient population served and the available mycological expertise. Certainly, CHROMagar will be useful as a primary culture medium for specimens such as oropharyngeal cultures from AIDS patients or surveillance stool and urine cultures from cancer or other immunocompromised patients. In these settings the detection and rapid presumptive identification of single or multiple yeast species may be quite important and have direct bearing on the selection of appropriate agents for antifungal therapy or prophylaxis. Although not endorsed as a definitive method for identification of yeast species, the accuracy of CHROMagar for identification of *C. albicans*, *C. tropicalis*, *C. krusei*, and *C.* (*Torulopsis*) *glabrata* in studies performed to date (1–3) would support its use in guiding species-specific treatment decisions. Given the fact that many laboratories do not perform identifications beyond a germ tube test, the use of CHROMagar would expand the level of mycological information available in many settings.

In summary, we offer additional evidence that CHROMagar is a very useful medium for use in medical mycology. Application of CHROMagar to both stock and clinical cultures demonstrates its ability to identify four *Candida* species: *C. albicans*, *C. tropicalis*, *C. krusei*, and *C.* (*Torulopsis*) *glabrata*. Taken together, these species accounted for almost 90% of the clinical yeast isolates colonizing patients in our surgical and neonatal ICUs. Given the significant differences in susceptibility to fluconazole and other azoles among these four species, a method that will allow for their simultaneous detection and identification may facilitate the appropriate use of antifungal agents in high-risk settings such as the surgical and the neonatal ICUs. Certainly, these capabilities plus the ability to detect mixed cultures of *Candida* spp. promise to improve and streamline the work flow in the mycology and clinical microbiology laboratory. Additional studies involving a broader variety of clinical specimens and *Candida* spp. as well as evaluations of the cost effectiveness and feasibility of CHROMagar use in various clinical and laboratory settings are certainly warranted.

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