Detection of Fifteen Species of *Candida* in an Automated Blood Culture System

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Candidemia was simulated with 15 *Candida* spp. by using an automated blood culture system. *Candida* growth was detected in 479/648 (74%) bottles: 211/216 (98%) aerobic bottles, 58/216 (27%) anaerobic bottles, and 210/216 (97%) mycology bottles. Only the growth of *Candida lipolytica* failed to be detected in all media.

*Candida* species are the fourth most common cause of nosocomial bloodstream infections in the United States (23). Historically accounting for greater than 75% of the cases of candidemia, *Candida albicans* now produces only about half of the cases of candidemia (1, 7, 20, 22, 23). At least 15 species of *Candida* have been reported to cause candidemia (12). Recovery of *Candida* from blood is commonly achieved by using standard bacterial blood culture media in automated blood culture systems (ABCSs) (2, 4, 5, 6, 14, 15, 22). Prior clinical and simulated candidemia studies have evaluated the abilities of these systems to detect the most common *Candida* species (2, 4, 5, 6, 8, 9, 10, 11, 14, 15, 17, 18, 21, 22), but limited or no data exist for the recovery of the rarer non-*C. albicans* *Candida* species. Our study examines the ability of one ABCS to recover the less common *Candida* spp. in a simulated model of candidemia.

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Six hundred forty eight BacT/ALERT (bioMérieux, Durham, NC) blood culture bottles were used in the study: 216 aerobic (FA) bottles, 216 anaerobic (SN) bottles, and 216 mycology (MB) bottles. Each bottle was first inoculated with fresh, whole, unpooled blood from 20 healthy volunteers.

The yeast inocula were then prepared according to the protocol of the Clinical and Laboratory Standards Institute (formerly NCCLS) (9, 10, 11, 16). The isolates studied included *C. albicans*, *Candida dubliniensis*, *Candida famata*, *Candida firmentaria*, *Candida glabrata*, *Candida guilliermondii*, *Candida inconspicua*, *Candida kefyr*, *Candida krusei*, *Candida lipolytica*, *Candida lusitaniae*, *Candida norvegensis*, *Candida parapsilosis*, *Candida rugosa*, and *Candida tropicalis*. Five isolates of each *Candida* species with the exception of *C. norvegensis* were tested; only two isolates of *C. norvegensis* were readily available. The isolates were obtained from our clinical laboratory and the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio. Suspensions of 10^2, 10^3, and 10^6 yeast cells/ml were prepared for each yeast isolate.

The bottles were then each inoculated with a 0.1-ml aliquot of the yeast suspension, resulting in final inocula of 10, 100, or 1,000 yeast cells. The mycology bottles were also inoculated with 1 ml of enrichment fluid, as recommended by the manufacturer. The Prepared bottles were immediately placed into the ABCSs, the BacT/ALERT 3D system (bioMérieux), and incubated at 35°C for up to 7 days. The bottles were removed when the machine detected growth or at the end of 7 days, if growth was not detected by the system. All bottles were subcultured when growth was detected or after 7 days to verify yeast viability (3, 19).

The independent variables of yeast species, inoculum, medium, and test (machine detection or terminal subculture) were compared with the dependent variables of time to detection and outcome (positive or negative) with three- and four-factor analyses of variance, followed by t tests corrected for multiple comparisons (SPSS software, version 13; SPSS Inc., Chicago, IL).

Growth was detected by the ABCS in 479/648 (74%) bottles (Table 1). All 479 bottles demonstrated yeast growth on terminal subculture. Of the 169 bottles without growth detected by the ABCS, terminal subculture demonstrated the growth of yeast in 161/169 (95%) bottles. All eight bottles without growth upon terminal subculture had been inoculated with *C. lipolytica*.

Growth was detected in 211/216 (98%) FA bottles and 210/216 (97%) MB bottles. All 11 without growth in these media had been inoculated with *C. lipolytica* isolates. Growth was detected in 58/216 (27%) SN bottles. *C. glabrata* and *C. albicans* were the only two species consistently detected in SN medium: 15/15 *C. glabrata* isolates and 13/15 *C. albicans* isolates. No growth of *C. famata*, *C. lipolytica*, *C. norvegensis*, or *C. parapsilosis* was detected in SN medium.

Three inoculum sizes were evaluated: 10, 100, and 1,000 yeast cells per bottle. More growth was detected with higher inocula: 10 yeast cells, 151/216 (70%); 100 yeast cells, 158/216 (73%); and 1,000 yeast cells 170/216 (79%).

The time to growth detection varied greatly, depending on the *Candida* spp., the medium, and the inoculum (Table 2). In general, growth was detected faster in FA and MB bottles than in SN bottles, and growth was detected more quickly with higher inocula. The mean time to detection of *C. albicans* was similar in all media at the same inoculum. The time to detection for most species was similar in FA and MB media.
ever, C. dubliniensis, C. parapsilosis, and C. rugosa were detected faster in FA medium than in MB medium (P < 0.01). MB medium detected the growth of C. lipo-lytica earlier than FA medium did. Most species demonstrated limited growth in SN medium. However, SN medium did allow significantly earlier growth detection than MB medium for C. glabrata, C. inconspicua, C. kefyr, and C. tropicalis (P < 0.01). C. glabrata was the only species that was detected significantly faster in SN medium than in FA medium (P < 0.001).

C. lipo-lytica was the only Candida species tested that was difficult to detect in the ABCS with all three of the media evaluated. C. lipo-lytica had the lowest rate of detection of any species (19/45 [42%]) and was not detected in anaerobic medium. Overall, 6/15 episodes of C. lipo-lytica candidemia would not have been detected. When C. lipo-lytica was detected, the time to growth detection was statistically (P < 0.001) slower than that for all other species, regardless of the medium or the inoculum size used. The lack of growth detection may be secondary to the fact that C. lipo-lytica can assimilate only a limited number of carbon sources (13). The clinical relevance of the poor detection of C. lipo-lytica is unknown, as this yeast rarely causes candidemia.

The use of mycology medium has been advocated by some authors to increase the rate of detection of candidemia. The use of MB medium improved the time to growth detection over the use of FA medium only for C. glabrata and C. lipol-
tica. MB medium is more expensive than FA and SN media and requires the addition of an enrichment fluid. Without a significant improvement in the rate of Candida detection or the time to detection, the routine use of this medium appears to be unnecessary.

Some authors advocate the use of only aerobic medium for standard blood cultures. The use of anaerobic medium in this study resulted in a clinically and a statistically significant earlier recovery of C. glabrata (P < 0.001) compared to the use of aerobic or mycology medium. Since C. glabrata causes as many as 25% of the candidemia episodes in the United States (20), it is essential that this organism be identified promptly.

Overall, the BacT/ALERT 3D ABCS has an excellent ability to detect candidal pathogens, even at low inocula, within a standard 5-day incubation period. These data suggest that the use of aerobic medium alone is adequate for the detection of most common and uncommon candidal pathogens.

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