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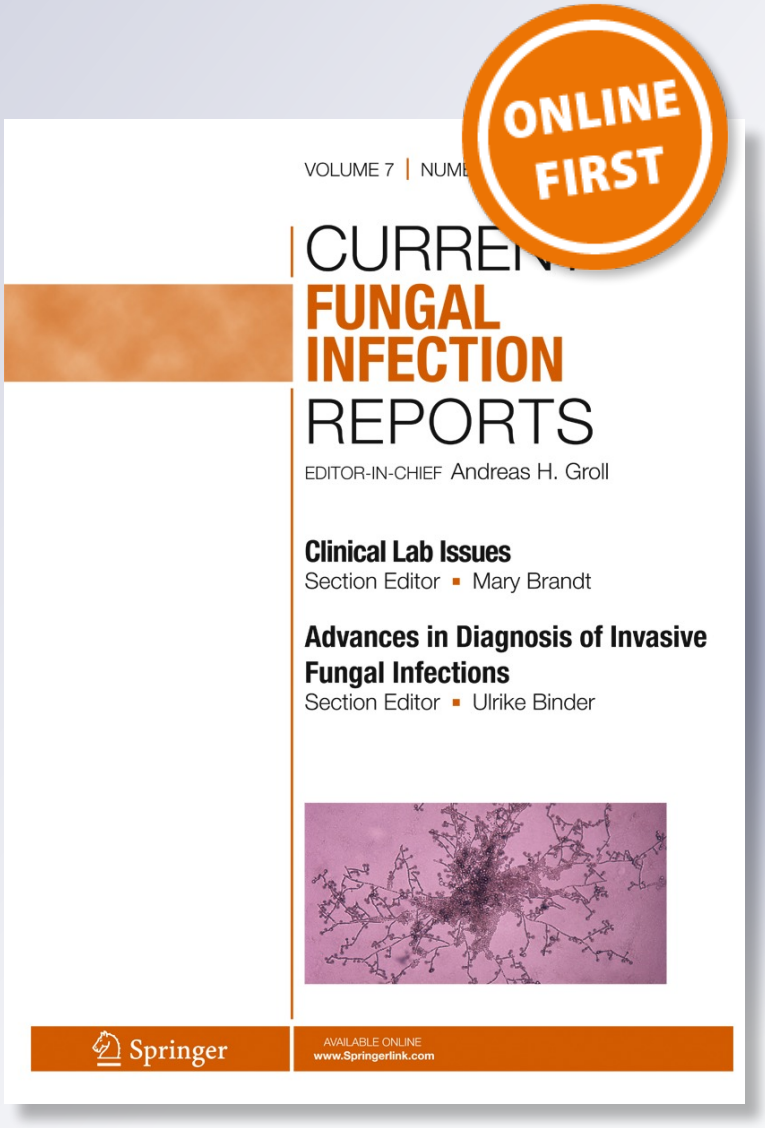

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Abstract Patients with invasive fungal infections still have high morbidity and mortality despite an increasing number of antifungals and other therapies. Because of this problem, an accurate and rapid diagnosis is mandatory in order to improve clinical outcome in these patients. In this paper we review the tools for the diagnosis of candidemia, including blood culture systems, chromogenic media, commercial kits for species identification, and newer technologies for the diagnosis of candidemia such as MALDI-TOF, PNA FISH and the T2 system.

Keywords Candidemia · Diagnosis · *Candida* · Blood culture

Introduction

Candidemia is a leading invasive fungal disease affecting hospitalized patients worldwide [1••, 2••]. It is one of the most frequent nosocomial bloodstream infections, and is associated with high mortality rates, especially in elderly patients and in those admitted to an intensive care unit (ICU) [3]. Part of the poor prognosis of candidemia is due to late diagnosis, resulting in a delay in starting appropriate antifungal therapy [4]. Therefore, early diagnosis is a key element in the management of candidemia. In this paper we review the tools available for the diagnosis of candidemia, including blood culture systems, chromogenic media, commercial kits and some new methods such as MALDI-TOF, PNA FISH and the T2 system.

Blood Cultures

Blood cultures have become one of the most important and frequently performed tests in the clinical microbiology laboratory [5]. Conventional blood culture methods involve visual examination of blood culture bottles for evidence of growth, and blind subculture on solid media. While the procedures of venting vacuum bottles and using a biphasic medium improve their performance [6, 7], conventional methods are suboptimal for the diagnosis of candidemia and should be discouraged. Likewise, although lysis centrifugation increases the recovery of *Candida* species from blood cultures, it is not practical for routine use in large hospitals [8, 9]. By contrast, automated blood culture methods are considered standard in microbiology laboratories for the diagnosis of candidemia. The two systems BACTEC and BactAlert have been widely tested.

In one study, the growth rate and time to positivity of aerobic/F bottles of the BACTEC 9120 system was evaluated in 11,156 blood samples from patients in the intensive care unit. Bottles were inoculated with 5 ml and 10 ml of blood from pediatric and adult patients, respectively. Yeasts were detected in 14 % of the bottles. The lowest mean time to positivity was for *Candida krusei* (18 h) and the highest was for *C. glabrata* (31 h) [10]. In another study, the BACTEC 9240 automated blood culture system was evaluated in simulated candidemia. Suspensions of 50 *Candida* isolates were prepared and aliquots containing 1,000 CFU were introduced into sets of BACTEC Plus culture bottles (one Aerobic/F and one Anaerobic/F, total 100 bottles) each containing 10 ml of blood. Growth was detected in 56 of the 100 bottles, with four isolates failing to grow in either bottle after 21 days of incubation. Most species grew quickly, except *C. glabrata* in the aerobic medium, which took 120 h to grow [11].

In a subsequent study by the same group, BACTEC and BacT/ALERT were directly compared. Aerobic, anaerobic,

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and mycology media for each system were inoculated with fresh blood from healthy donors plus 1,000 yeasts of 50 isolates of *Candida* species. *Candida* was detected in 90 % of the BacT/ALERT bottles and in 66 % of the BACTEC bottles. Growth was detected in all BacT/ALERT and BACTEC mycology bottles, and all BacT/ALERT aerobic bottles. Among 65 negative bottles, 50 were from the BACTEC system (5 aerobic and 45 anaerobic) and 15 from the BacTALERT (all anaerobic). The mean time to growth detection was 25 h in the BacT/ALERT system and 27 h in the BACTEC system. The authors concluded that if specialized mycology bottles are used, both systems perform equally well, but if aerobic media are used, the Bact/ALERT is better than the BACTEC system [12].

The good performance of the aerobic Bact/ALERT bottles was confirmed in a study that tested 15 *Candida* species in 216 aerobic bottles, 216 anaerobic bottles and 216 mycology bottles, which were inoculated with suspensions containing different concentrations of yeasts. Growth was detected in 98 % of aerobic bottles, 97 % of mycology bottles and 27 % of anaerobic bottles. The time to growth detection was similar in aerobic and mycology bottles for most species, with the exception of *C. dubliniensis*, *C. parapsilosis* and *C. rugosa*, which were detected earlier in the aerobic bottles, and *C. glabrata* and *C. lipolytica*, which grew more quickly in the mycology bottles. The authors concluded that the use of aerobic Bact/ALERT alone is adequate for the detection of most *Candida* pathogens [13]. However, in the presence of concomitant bacterial infection, the performance of the mycology bottles is superior to that of the aerobic bottles, as shown in two studies [14, 15•].

The effect of the use of blind subcultures in automated blood culture systems was evaluated in a retrospective study. A total of 2,154 blood cultures from 285 patients processed by the BacT/ALERT were selected for subculture on day 3 of incubation. Candidemia was diagnosed in 52 patients, and *Candida* species grew from subcultures from 14 patients, 11 of whom had already been diagnosed before subculture. The three patients diagnosed by subculture represented only 1.1 % of the 285 patients [16].

While the performance of different automated blood culture systems and bottles have been extensively evaluated, little is known about the effect of the volume of blood on the growth of *Candida* species from blood cultures. Most of the recommendations are extrapolated from studies with bacteria. One study evaluated the BACTEC system and showed that the recovery rates of both bacteria and fungi were higher if the blood volume was ≥5 mL [17]. In neonates a comparison between one and two bottles showed no advantage in using two bottles in the rate of growth detection of *Candida* species [18].

It is important to consider that when bacteria are mixed with yeasts in the bottles, recovery of the yeasts decreases. In such cases, mycology bottles should be used [14].

Yeast Identification

The diagnosis of candidemia involves two important steps: growth detection and species identification. As pointed out, the time to growth detection of some *Candida* species such as *C. glabrata* is longer. A further complication is that the time to species identification may also be longer, compared with *C. albicans*. For example, one study showed that the mean time to growth detection was 35 h for *C. albicans* and 80 hours for *C. glabrata*, and the mean time to final identification was 85 h for *C. albicans* and 154 h for *C. glabrata* [19]. Therefore, species identification is also a critical step in the proper management of candidemia.

Conventional methods for species identification include the isolation of the yeast in a routine medium such as among others Sabouraud dextrose agar or yeast medium. For species identification, the germ tube test is easy to perform and allows *C. albicans* (germ tube positive) to be distinguished from and non-albicans species within 3 h, with the exception *C. dubliniensis*, which also produces a germ tube. False-negative results may occur in 5–10 % of cases.

In chromogenic media, colonies of yeasts show different colors that differentiate some species of the genera *Candida*. These media are particularly useful for diagnosing infection caused by more than one species. In the CHROMagar Candida medium, colonies of yeasts show different colors after 48 h of incubation at 37 °C. *Candida albicans* (but also *C. dubliniensis*) show as green, *C. tropicalis* as blue and *C. krusei* as pink and fuzzy. Other *Candida* species can be white to pink but not specific for species identification [20]. Another chromogenic medium is Agar Candida ID. In a comparison with CHROMagar Candida, Agar Candida ID allowed easier identification of *C. albicans* [21].

Candida albicans and *C. dubliniensis* can be differentiated using different tests, such as the formation of rough colonies on Niger seed agar [22], sunflower seed (*Helianthus annuus*) agar [23] and tobacco agar [24], and the production of dark green colonies on CHROMagar Candida and the inability to grow in a hypertonic medium containing NaCl [25]. In addition to these methods, molecular typing and MALDI-TOF may help in the differentiation of these two *Candida* species [26, 27].

Various commercially available kits do exist for the identification of yeasts [28–31]. Recently, three different commercial kits were evaluated: API 32C, Auxacolor and Vitek2-YST. Among 253 isolates previously identified by molecular techniques, species were correctly identified in 84 % of isolates with Vitek 2-YST, 83 % with the API ID32C, and 80 %

with the Auxacolor. Considering the most common yeasts causing invasive disease (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. tropicalis* and *Cryptococcus neoformans*), the three systems demonstrated comparable performance: 94.5 % with the Auxacolor system, 94 % with API ID32C and 91 % with Vitek 2-YST. By contrast, for infrequent yeasts, such as *C. dubliniensis*, *C. famata*, *C. kefyr* and *C. guilliermondii*, Vitek 2-YST performed best (correct identification in 64 %), followed by API ID32C (56 %) and Auxacolor (43 %). None of the systems was able to discriminate the different species within the *C. parapsilosis* complex (*C. parapsilosis*, *C. metapsilosis* and *C. orthopsilosis*), or the *C. glabrata* complex (*C. bracarensis* and *C. nivariensis*) [32•].

New Methods

In addition to conventional methods of species identification, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been developed. This procedure has been increasingly used for the identification of bacteria [33, 34] and fungi [35–38]. In general, the procedure is very accurate, and useful for identifying species that cannot be identified by conventional methods, including species within a complex [39].

Schubert et al. have demonstrated the usefulness of MALDI-TOF in the identification of yeasts and bacteria directly from positive blood cultures. Of yeasts from positive blood cultures, identification at the species level was achieved in 70.6 %, including *C. albicans*, *C. glabrata*, *C. guilliermondii*, and *C. parapsilosis* [40]. In another study, the authors found that one colony picked up from a chromogenic agar medium is sufficient to prepare the sample for analysis by MALDI-TOF. From a total of 183 isolates 95.1 % were identified within 48 h and 96.2 % within 72 h [41]. Although MALDI-TOF seems to be a good system for fast yeast identification, not 100 % of the isolates are identified.

Another system for species identification is peptide nucleic acid fluorescent in situ hybridization (PNA-FISH). This method identifies fungi by using a PNA probe [42–44]. A study compared the time to identification of bacteria and *Candida* from blood cultures using conventional cultures and PNA-FISH. The average time to identification was 83.6 h with the standard culture methods and 11.2 h with PNA-FISH. The overall accuracy of PNA-FISH was 98.8 % [45].

A new system for rapid detection of *Candida* directly from the whole blood has been recently developed. The system uses PCR, followed by hybridization of the amplified DNA to capture probe-decorated nanoparticles. The hybridization yields nanoparticle microclusters that cause changes in T2 magnetic resonance signal, which is detected by a device. With this technique, *Candida* can be detected in whole blood in 3 h [46]. The system was tested in comparison with the BACTEC 9050 using Aerobic Plus/F bottles in seeded blood samples at concentrations of fungi between 3.1 and 11 CFU/ml, testing *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. krusei*. The BACTEC detected *Candida* growth in 100 % of bottles within 5 days, with the exception of *C. glabrata* (no detection). The T2 system had a 100 % detection rate for all species within 3 h [47].

Conclusions

The diagnosis of candidemia relies on blood culture. The two widely used systems, BACTEC and BacT/ALERT, have similar performances when fungal bottles are used. However, if aerobic bottles are used, the performance of the BacT/ALERT is superior, especially for some *Candida* species such as *C. glabrata*. The time to positivity for *C. glabrata* is usually longer. For species identification, chromogenic media are very helpful, and the different commercial kits offer quite similar results, although species identification may take 2–3 days. Newer technologies such as MALDI-TOF, PNA-FISH and T2 are faster and they seem promising for routine use.

Compliance with Ethics Guidelines

Conflicts of interest Roxana Vitale declares no conflicts of interest.

Marcio Nucci has received consulting fees and payment for speaking or development of educational presentations from Pfizer, Merck, Astellas, and Gilead; and is on the board of Pfizer, Merck, Gilead and Astellas.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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