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Recent Progress in the Diagnosis of Pathogenic *Candida* Species in Blood Culture

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Abstract Candidemia has become an emerging invasive fungal disease. Prompt treatment with appropriate antifungal agent is crucial to reduce the mortality of candidemia. The conventional blood culture method, which is considered the gold standard for candidemia diagnosis, has a low sensitivity and is time-consuming to perform. Recently, several novel advanced diagnostic methods that have a higher sensitivity and a shorter turnaround time than the conventional blood culture method have been developed for the early detection of *Candida* in blood samples or in blood culture broth. Most of these newer methods were developed using various molecular techniques, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, peptide nucleic acid fluorescence in situ hybridization, and a number of DNA-based techniques including in-house and commercial polymerase chain reactions. In this article, we review and summarize the novel molecular methods that have been recently used for the detection and identification of *Candida* organisms in blood specimens.

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Introduction

Invasive candidiasis has become an emerging and difficult-to-treat fungal infection in clinical practice, mainly because of the increased use of novel and potent immunosuppressive agents, the advances in chemotherapy for solid and hematologic malignancies, and the increase in the number of organ transplant recipients [1, 2]. Although neutropenic patients are the biggest group at risk for candidemia, *Candida* species are one of the most common etiologic agents of bloodstream infection in non-neutropenic patients who are admitted to intensive care units [3].

Preemptive and empirical antifungal therapies for invasive candidiasis are based on the clinical settings for each patient, including the patient's infection severity and history of antifungal drug use as well as the epidemiological data from the institution that is treating the patient. Candidemia mortality is as high as 40 % and is associated with a lack of appropriate antifungal therapy [4, 5]. Recent clinical data have shown that the prevalence of non-*albicans Candida* species, in particular *Candida glabrata* and *Candida krusei*, is increasing. These species exhibit more antifungal resistance, especially to the azole antifungal agents [6]. Rapid identification and speciation of

Candida from blood culture are therefore crucial for prompt species-appropriate antifungal therapy. Unfortunately, the conventional blood culture technique for identifying *Candida* species has a low sensitivity (approximately 50 %) and is a time-consuming process for detecting candidemia [7, 8].

Evolution of *Candida* Diagnosis

Speciation of *Candida* can be achieved by conventional methods based on the phenotypic characteristics of the fungus. Additionally, the *Candida* germ tube test and chlamydospore formation have both been used for the identification of *C. albicans*. However, carbohydrate assimilation and fermentation tests are currently the standard methods for species differentiation because different *Candida* species exhibit different biochemical patterns. Many automated biochemical and assimilation tests, such as API® Candida systems (bioMerieux) and VITEK®2 YST ID card (bioMerieux), have been developed to reduce the turnaround time from more than 48 to 15–24 h [9]. However, these methods are still time-consuming as they require isolated fungal colonies grown on solid media. Furthermore, species identification can be inaccurate in some closely related *Candida* species [10]. The misidentification of *Candida* species by this method is generally due to the variation of phenotypic characteristics for each species or the presence of mixed *Candida* species within the isolates.

Chromogenic candida media, such as commercially prepared CHROMagar™ Candida medium and CandiSelect® 4 agar (BioRad), have been widely used for *Candida* speciation [9]. These agars contain chromogenic substrates that react with an enzyme secreted from specific *Candida* species to produce specific color reactions on the media. The sensitivities and specificities of these media are quite high for *C. albicans, C. tropicalis,* and *C. krusei,* but inaccuracies often occur due to the variation of enzymatic reactions within the same species, especially species other than *C. albicans* and *C. tropicalis*.

The detection of beta-D-glucan is a non-culture-based method in which beta-D-glucan is used as a surrogate marker to diagnose invasive candidiasis, including candidemia. However, the sensitivity and specificity of this method are not very high [11], and false-positive results have been reported owing to potential source contamination with contaminants such as human immunoglobulin, gauze that contains glucan, or cellulose membrane from hemodialysis [12].

Recent advanced technologies have been developed that aim to increase the sensitivity and specificity of detection and reduce the turnaround time for candidemia diagnosis. These technologies include new advanced methods for rapid *Candida* identification directly from blood culture broth, which removes the need for further subculturing on specific solid media. Furthermore, some methods can directly identify *Candida* from a patient's whole blood samples. These advanced methods include spectroscopy-based methods, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), peptide nucleic acid fluorescence in situ hybridization (PNA-FISH), and DNA-based molecular techniques. Each method provides different advantages and limitations. This review will focus on describing the principle behind each of these tests as well as their benefits, limitations, sensitivities, and specificities. A summary and comparison of each method are shown in Table 1.

Spectroscopy

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS)

Currently, MALDI-TOF/MS is frequently used for the identification of pathogenic bacteria and fungi at the species or genus level using specimens from pathogen-positive blood cultures or colonies on solid media. However, this technique can also be applied directly to clinical specimens. MALDI-TOF/MS is based on the detection of protein molecules by calculating their mass-to-charge values and matching them with organisms in a specific database. Previous studies showed that the accuracy of this technique for yeast identification is extremely high and well correlated with the organisms at the genus and species levels, compared with conventional methods. Sensitivities of MALDI-TOF/MS in detecting *Candida* spp. have been reported from 91.3 to 100 % with a very high specificity (100 %) [13–16]. However, *C. guilliermondii* is frequently misidentified [15]. The advantages of this

Table 1 Advanced *Candida* diagnostic methods

Test	Method	Specimen	*Candida* species	Turnaround time	Sensitivity (%)	Specificity (%)
MALDI-TOF/MS	Mass spectroscopy	Blood culture broth	Varies (depends on the database)	90 min	91.3–100	100
PNA-FISH	Spectroscopy (26sRNA)	Blood culture broth	First generation Five common spp. *C. albicans/C. parapsilosis* *C. tropicalis* *C. glabrata/C. krusei*	90 min	97.5–98.9	98.2–100
			Second generation Three common spp. *C. albicans* *C. parapsilosis* *C. glabrata*	30 min	99.7	98
In-house PCR	PCR Nested PCR RT-PCR	Whole blood or serum	Varies (depends on the technique)	4–12 h	95	92
Prove-it^{TM} Sepsis	Microarray	Blood culture broth	Eight common spp. *C. albicans* *C. tropicalis* *C. glabrata* *C. krusei* *C. parapsilosis* *C. guillermondii* *C. lusitaniae* *C. dublinensis*	3 h	99	98
Candida 7-plex	Luminex	Blood culture broth	Seven common spp. *C. albicans* *C. tropicalis* *C. glabrata* *C. krusei* *C. parapsilosis* *C. guillermondii* *C. lusitaniae*	5 h	100	99–100
Lightcycler SeptiFast	DNA-based (ITS1)	Whole blood or blood culture broth	Five common spp. *C. albicans* *C. tropicalis* *C. glabrata* *C. krusei* *C. parapsilosis*	6 h	61 (Whole blood) 95 (Blood culture broth)	99
T2 magnetic resonance assay	DNA-based	Whole blood	Five common spp. *C. albicans/C. tropicalis* *C. parapsilosis* *C. glabrata/C. krusei*	4 h	91–100	97.8–99

test are the shorter turnaround time for *Candida* identification, which is approximately 90 min for specimen processing after obtaining a fungus-positive blood culture, and its capability to detect rare *Candida* species [17]. The limitations of this method include its high setup cost, requirement for a useful database, low sensitivity for direct testing on whole blood samples (due to protein contamination), and potential misidentification of samples from polyfungal bloodstream infections.

Peptide Nucleic Acid Fluorescence In Situ Hybridization (PNA-FISH)

The PNA-FISH technique is a molecular method that uses dual color-labeled fluorescent DNA probes to target the 26S rRNA sequences of *Candida* species and detect the fluorescent colors under a fluorescence microscope. Different *Candida* species are identified by different colors. To date, there have been two generations of the PNA-FISH method. The first generation of yeast PNA-FISH is the Yeast Traffic Light PNA-FISH identification system (AdvanDx, Woburn, USA), which can distinguish the five most common *Candida* species directly from fungus-positive blood cultures. The turnaround time for this test is about 90 min, and it can identify *C. albicans, C. tropicalis, C. glabrata, C. krusei*, and *C. parapsilosis*. In this test, *C. albicans* and *C. parapsilosis* fluoresce green, *C. tropicalis* fluoresces yellow, and *C. glabrata* and *C. krusei* fluoresce red. Other *Candida* species and other fungi are not identified by the probes, so they do not fluoresce any color. Although this test cannot distinguish *C. albicans* from *C. parapsilosis* or *C. glabrata* from *C. kruesi*, it provides enough information for physicians to make a preliminary decision about the likely fluconazole susceptibility of the species; green fluorescence may suggest fluconazole-susceptible *Candida*, while red or yellow fluorescence may suggest a fluconazole-resistant species [18].

The second-generation yeast PNA-FISH is the PNA *Candida* QuickFISH BC (AdvanDx, Woburn, USA). This test can be used to distinguish among three common *Candida* species: *C. albicans*, *C. glabrata*, and *C. parapsilosis*. In this test, *C. albicans* fluoresces green, *C. glabrata* fluoresces red, and *C. parapsilosis* fluoresces yellow. Its turnaround time is approximately 30 min, which is faster than that for the first-generation test [19].

Sensitivities of 97.5–98.9 % have been reported for the first-generation tests, with specificities ranging from 98.2 to 100 % [18, 20, 21]. The sensitivity and specificity of the second-generation test are 99.7 and 98.0 %, respectively [19]. However, these assays have several limitations. They are expensive assays to perform, and species misidentification is possible in samples with mixed *Candida* infection. Additionally, the first-generation test cannot identify *Candida* into a single species, and neither of these tests is unable to identify rare *Candida* species. Finally, these tests require well-trained technicians to interpret the results using fluorescence microscopy.

DNA-Based Methods

In-House DNA-Based Methods

DNA-based methods have been developed that involve sequencing of DNA regions that are highly conserved in *Candida*. Common sequences used in these methods include 5.8S, 18S, 26S, and 28S ribosomal RNA as well as the internal transcribed spacer (ITS) regions, especially ITS1 and ITS2.

There are several techniques that use DNA-based methods. One of the most commonly applied methods is polymerase chain reaction (PCR), which includes standard PCR, nested PCR, and real-time PCR. The PCR processing times range from 4 to 12 h, and the relatively shorter turnaround time for this method is one of its advantages. Most newly developed in-house PCR methods use real-time PCR assays, even though they are more expensive than traditional PCR assays, because they are more convenient and have a shorter turnaround time [22–24]. Microarrays are another DNA-based method that can be used to detect *Candida* in blood culture. However, they have not been widely used in routine clinical practice because this technique is complicated and labor intensive [25]. Pyrosequencing methods have also been used in some laboratories [26], and another method used to identify *Candida* has been developed based on amplified fragment length polymorphisms (AFLPs) [27]. Given the variety of DNA-based methods that have been developed for *Candida* identification, each of which has a different range of sensitivities and specificities, it is difficult to evaluate the overall sensitivity and specificity of DNA-based methods. However, a recent meta-

analysis by Avni and colleagues has shown that the pooled sensitivity and specificity of PCR-based methods were 95 and 92 %, respectively, in patients with suspected invasive candidiasis [28].

Commercial DNA-Based Methods

Currently, there are several commercial DNA-based methods available for *Candida* identification and speciation. These methods have been designed to decrease the turnaround time.

Prove-it™ Sepsis

Prove-it™ sepsis (Mobidiag Ltd, Finland) is a DNA-based identification method using PCR amplification followed by specific identification on a microarray. It provides identification from blood culture broth of eight common *Candida* species: *C. albicans*, *C. tropicalis, C. glabrata, C. parapsilosis*, *C. krusei*, *C. guilliermondii*, *C. lusitaniae*, and *C. dublinensis* [25]. Its sensitivity and specificity are 99 and 98 %, respectively, and its turnaround time is approximately 3 h.

Candida 7-Plex Panel

The Candida 7-plex panel, developed by Luminex Molecular Diagnostics (Toronto, Canada), is a multiplex PCR using xMAP technology for amplification and detection. It can distinguish between seven different *Candida* species: *C. albicans*, *C. tropicalis, C. glabrata, C. parapsilosis*, *C. krusei*, *C. guilliermondii*, and *C. lusitaniae*. This test can identify *Candida* species growing in blood culture broth or from fungal colonies, and its turnaround time is approximately 5 h, which is longer than that for PNA-FISH. The sensitivity and specificity for detecting *Candida* in blood culture broth is 100 and 99–100 %, respectively [29, 30].

Lightcycler SeptiFast

Lightcycler SeptiFast (LC-SF; Roche Diagnostics, Mannheim, Germany) is a real-time multiplex PCR test that is able to detect multiple genera of bacteria and fungi, including the five most common *Candida* species: *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis*, and *C. krusei*. This test was developed to amplify multi-copy target regions from ITS between the 18S and 5.8S subunits of fungal ribosomal RNA, and it uses a highly specific melting point analysis. The sensitivity threshold for detection is 100 colony-forming units (CFU)/mL for *C. glabrata* and 30 CFU/mL for the others. The turnaround time of this test is approximately 6 h [31]. LC-SF has a very high specificity (99 %) but a low sensitivity (61 %) [32].

T2 Magnetic Resonance Assay

Another novel molecular method for *Candida* identification is the T2 magnetic resonance assay (T2Biosystems, USA), which uses whole blood PCR amplification followed by magnetic resonance detection. The test is able to detect five common *Candida* species, but it is unable to distinguish *C. albicans* from *C. tropicalis* or *C. glabrata* from *C. krusei*. The sensitivity threshold of this test is 1 CFU/mL for *C. tropicalis* and *C. krusei*, 2 CFU/mL for *C. albicans* and *C. glabrata*, and 3 CFU/mL for *C. parapsilosis*, which are all lower than those of LC-SF. The turnaround time of the T2 magnetic resonance assay is approximately 4 h, and it has a higher sensitivity (88–100 %) than LC-SF, but a similar specificity (97.8–99 %). However, the sensitivity varies among the species; it is highest in *C. parapsilosis* (94 %), followed by *C. albicans*/*C. tropicalis* (92 %), and *C. glabrata/C. krusei* (88 %) [33, 34].

Conclusion

Candidemia is an important emerging disease that is associated with a high mortality so prompt antifungal therapy is crucial. Because the lack of sensitivity of the previous gold standard technique, fungal blood culture, may lead to a delay in commencing antifungal therapy, recent molecular methods with varying sensitivities and specificities have been developed for the direct detection and identification of *Candida* directly from blood samples or from blood culture broths. Despite these advances, the currently available molecular methods that can detect *Candida* directly from blood samples are limited, and these techniques are not able to provide antifungal susceptibility. Therefore, the development of new advanced diagnostic methods is still warranted.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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