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Multicenter Evaluation of *Candida* QuickFISH BC for Identification of *Candida* Species Directly from Blood Culture Bottles

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Candida* species are common causes of bloodstream infections (BSI), with high mortality. Four species cause >90% of *Candida* BSI: *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. Differentiation of *Candida* spp. is important because of differences in virulence and antimicrobial susceptibility. *Candida* QuickFISH BC, a multicolor, qualitative nucleic acid hybridization assay for the identification of *C. albicans* (green fluorescence), *C. glabrata* (red fluorescence), and *C. parapsilosis* (yellow fluorescence), was tested on Bactec and BacT/Alert blood culture bottles which signaled positive on automated blood culture devices and were positive for yeast by Gram stain at seven study sites. The results were compared to conventional identification. A total of 419 yeast-positive blood culture bottles were studied, consisting of 258 clinical samples (89 *C. glabrata*, 79 *C. albicans*, 23 *C. parapsilosis*, 18 *C. tropicalis*, and 49 other species) and 161 contrived samples inoculated with clinical isolates (40 *C. glabrata*, 46 *C. albicans*, 36 *C. parapsilosis*, 19 *C. tropicalis*, and 20 other species). A total of 415 samples contained a single fungal species, with *C. glabrata* ($n = 129$; 30.8%) being the most common isolate, followed by *C. albicans* ($n = 125$; 29.8%), *C. parapsilosis* ($n = 59$; 14.1%), *C. tropicalis* ($n = 37$; 8.8%), and *C. krusei* ($n = 17$; 4.1%). The overall agreement (with range for the three major *Candida* species) between the two methods was 99.3% (98.3 to 100%), with a sensitivity of 99.7% (98.3 to 100%) and a specificity of 98.0% (99.4 to 100%). This study showed that *Candida* QuickFISH BC is a rapid and accurate method for identifying *C. albicans*, *C. glabrata*, and *C. parapsilosis*, the three most common *Candida* species causing BSI, directly from blood culture bottles.

Candida species are ranked as the third or fourth most common etiologic agents associated with nosocomial bloodstream infections (1, 2). Mortality rates are estimated to be as high as 45% (3, 4), due in part to slow diagnostic methods and to inappropriate initial antifungal treatment (5, 6). *Candida albicans* and other yeasts reside as commensals of the skin and mucous membranes of the gastrointestinal and genitourinary tracts, causing infection when the host becomes debilitated or immunocompromised. Risk factors for invasive candidiasis include central venous catheters, parenteral nutrition, organ transplantation, hemodialysis, surgery, burns, long-term stay in an intensive care unit, and previous administration of broad spectrum antimicrobial and immunosuppressive agents (7, 8).

The most common *Candida* species found in healthy individuals include *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* (9). These species cause more than 90% of invasive infections (10). However, the prevalence of these different *Candida* species varies widely geographically, and their antifungal susceptibilities are species specific (11). Consequently, rapid, accurate identification of the causative *Candida* species is critical for successful treatment.

Currently, blood culture is the gold standard method for the diagnosis of fungal bloodstream infection (12). When growth is detected, blood culture medium is drawn for Gram stain and subculture on agar plates for subsequent phenotypic identification (13), which typically takes at least 72 h (14). To overcome this relatively long turnaround time of identification, several molecular methods have been evaluated for identification of yeasts directly from blood culture bottles such as real-time PCR, matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry, and peptide nucleic acid fluorescence *in situ* hybridization (PNA-FISH) (15, 16). A method to detect *Candida* directly from patient's blood specimens within 3 h has also been developed (17, 18).

The first generation of yeast PNA-FISH (AdvanDx, Woburn, MA) distinguishes between the five commonest *Candida* species directly from positive blood cultures within 90 min. The method uses fluorescence-labeled probes to complement species-specific rRNA sequences: *C. albicans* and *C. parapsilosis* fluoresce green, *C. tropicalis* fluoresces yellow, and *C. glabrata* and *C. krusei* fluoresce red. The assay cannot distinguish between *C. albicans* and *C. parapsilosis* or between *C. glabrata* and *C. krusei* (15). A second-generation yeast PNA-FISH, PNA *Candida* QuickFISH BC (AdvanDx), is faster (30 min) and able to differentiate between the most common *Candida* spp.: *C. albicans* fluoresces green, *C. glabrata* fluoresces red, and *C. parapsilosis* fluoresces yellow, while other yeasts do not produce any fluorescence.

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TABLE 1 Numbers of clinical and contrived samples tested from each study site

Study site	No. of samples	
	Clinical	Contrived
Case Western Reserve University	32	NA[a]
Cedars Sinai Medical Center	21	41
Columbia University Medical Center	37	39
Detroit Medical Center	25	NA
Indiana University	65	38
Johns Hopkins Hospital	47	3
University of Virginia	31	40
Total	258	161

[a] NA, not applicable.

TABLE 2 Distribution of clinical and contrived blood culture bottle types studied

Blood culture system and bottle type[a]	No. of bottles studied		
	Clinical	Contrived	Total
BacT/Alert			
Aerobic	53	38	91
Anaerobic	10	2	12
Bactec			
Aerobic	154	104	258
Anaerobic	31[b]	17	48
Pediatric	10	0	10
Total	258	161	419

[a] All bottles were standard bottles unless indicated otherwise.
[b] Four of these bottles were Bactec Anaerobic Plus bottles.

The *C. albicans* probe is species specific and does not react with *C. dubliniensis*. Similarly, the *C. parapsilosis* probe does not react with *C. orthopsilosis* or *C. metapsilosis*. However, the *C. glabrata* probe does react with *C. bracarensis* and *C. nivariensis* (16).

In the present study, we used a prototype lot of PNA *Candida* QuickFISH BC (AdvanDx). *Candida* QuickFISH BC is a FISH method using peptide nucleic acid (PNA) probes designed to detect specific rRNA sequences of yeast species on microscope slides from positive blood culture bottles. Fluorophore-labeled PNA probes that target unique rRNA sequences and quencher probes that target unbound PNA probes are added to smears and hybridized at 55°C for 15 min. PNA probes that do not bind to target rRNA sequences will bind to a quencher probe to form a nonfluorescent probe-quencher complex. Routinely, bloodstream infections caused by yeast are presumptively identified by Gram stain from positive blood cultures, with final identification and differentiation requiring subculture and biochemical analysis, which can take several days. We used *Candida* QuickFISH BC to identify *Candida* species directly from positive blood culture bottles in a multicenter study at seven institutions.

MATERIALS AND METHODS

Samples from clinical and contrived blood culture bottles were tested at seven study sites with a prototype lot of *Candida* QuickFISH BC. Clinical samples were obtained from routine blood cultures at all seven study sites. Testing was performed on bottles that signaled positive on automated blood culture devices and showed yeast by Gram stain within 48 h of bottles signaling positive. Contrived samples were produced at five study sites using simulated blood cultures, with aliquots of blood broth medium (using 10 ml of blood per bottle) inoculated with fresh subcultures of clinical isolates. These were incubated with shaking at 35 to 37°C until yeast were visible upon Gram stain, which in most cases was within 24 h.

Candida QuickFISH BC was performed according to the manufacturer's directions. Briefly, 10 μl of fluid from a positive blood culture bottle and one drop of QuickFix-1 were mixed on the sample area of the QuickFISH slide, left to dry (1 to 3 min), and then 2 drops of QuickFix-2 were added and allowed to dry for 1 min. One drop each of *Candida* PNA blue and *Candida* PNA yellow were mixed on a coverslip, which was placed onto the QuickFISH slide that contained the fixed blood sample, followed by incubation for 15 min at 55°C using the supplied heat block. The slides were then examined by fluorescence microscopy using appropriate filters. Green fluorescence indicates *C. albicans*, red fluorescence indicates *C. glabrata*, and yellow fluorescence indicates *C. parapsilosis*, whereas no fluorescence indicates a yeast other than *C. albicans*, *C. glabrata*, or *C. parapsilosis*.

The QuickFISH BC results were compared to conventional identification (germ tube test, growth or morphology on fungal media, and identification by API 20C, Phoenix, or Vitek yeast biochemical card, as appropriate). Since conventional identification systems may not distinguish *C. parapsilosis* from *C. orthopsilosis* and *C. metapsilosis*, samples identified by conventional identification as *C. parapsilosis* were submitted for sequence analysis to distinguish between these species.

RESULTS

A total of 419 yeast-positive blood culture bottles were studied at the seven study sites, consisting of 258 clinical samples and 161 seeded samples (Table 1). Blood culture bottle types included BacT/Alert aerobic and anaerobic bottles and Bactec aerobic, anaerobic, and pediatric bottles (Table 2). The distribution of yeast species in the clinical and contrived samples is shown in Table 3. Sixty-five samples initially identified by conventional testing as *C. parapsilosis* were sequenced, and the results were as follows: 59 *C. parapsilosis*, 3 *C. metapsilosis*, 2 *C. orthopsilosis*, and 1 *C. tropicalis* sample.

A total of 415 samples contained a single fungal species, with *C. glabrata* ($n = 129$; 30.8%) being the most common isolate, followed by *C. albicans* ($n = 125$; 29.8%), *C. parapsilosis* ($n = 59$; 14.1%), *C. tropicalis* ($n = 37$; 8.8%), and *C. krusei* ($n = 17$; 4.1%). Four clinical samples contained two fungal species: *C. albicans* and *C. glabrata* ($n = 2$), *C. glabrata* and *C. tropicalis* ($n = 1$), or *C. albicans* and *C. parapsilosis* ($n = 1$). Of the 258 clinical specimens studied, it was noted that 217 were in aerobic and 41 in anaerobic bottles. It was further noted that the species distribution between aerobic and anaerobic bottle types differed, with 39.3% of the 89 *C. glabrata* versus 3.6% of the 169 other yeast species present in the anaerobic bottles ($P < 0.0001$). *C. glabrata* was found in 85.4% (35/41) of the clinical anaerobic bottles.

The overall agreement between conventional identification and QuickFISH BC was 99.3% (416/419) (Table 4). Agreement was 100% for *C. albicans* ($n = 125$), *C. glabrata* ($n = 129$) and mixed *Candida* species ($n = 4$). Agreement was 98.3% for *C. parapsilosis* (58/59), with no fluorescence detected in the one discrepant sample. There was 98.0% agreement (100/102) for other yeast species, with yellow fluorescence in the two discrepant samples, one of which contained *C. metapsilosis* and the other of which contained *C. tropicalis*. The overall sensitivity was 99.7%, and the specificity was 98.0%.

TABLE 3 Identification of the yeast species present in the 419 samples studied[a]

Yeast species[a]	No. of samples		
	Clinical	Contrived	Total (%)
Candida glabrata	89	40	129 (30.8)
Candida albicans	79	46	125 (29.8)
Candida parapsilosis	23	36	59 (14.1)
Candida tropicalis	18	19	37 (8.8)
Candida krusei	12	5	17 (4.1)
Candida guilliermondii	7	1	8 (1.9)
Candida lusitaniae	3	4	7 (1.7)
Candida dubliniensis	3	3	6 (1.4)
Candida metapsilosis	2	3	5 (1.2)
Candida orthopsilosis	3	1	4 (1.0)
Candida kefyr	2	1	3 (0.7)
Candida auris	1		1 (0.2)
Candida famata	1		1 (0.2)
Candida rugosa	1		1 (0.2)
Candida zeylanoides		1	1 (0.2)
Candida species, mixed[a]	4		4 (1.0)
Cryptococcus neoformans	6		6 (1.5)
Exophilia dermatitidis	1		1 (0.2)
Malassezia pachydermatis	1		1 (0.2)
Rhodotorula spp.	1		1 (0.2)
Saccharomyces cerevisiae	1	1	2 (0.5)
Total	258	161	419 (100)

[a] For the *Candida* species used in the study, the numbers of clinical and contrived samples and the percentage of each *Candida* species are shown.
[b] That is, *C. albicans*/*C. glabrata* ($n = 2$), *C. glabrata*/*C. tropicalis*($n = 1$), and *C. albicans*/*C. parapsilosis* ($n = 1$).

DISCUSSION

Currently, blood culture is the gold standard method for diagnosis of fungal bloodstream infections (12). When growth is detected, blood culture medium is sampled for Gram stain and subculture on agar plates for subsequent phenotypic identification (13), which typically takes at least 72 h (14). To overcome this relatively long turnaround time of identification, several molecular methods have been evaluated for identification of *Candida* species directly from blood culture bottles, such as real-time PCR (19, 20), multiplex-tandem PCR (21), PCR–high-resolution derivative melt analysis (22), PCR-electrospray ionization mass spectrometry (23), DNA microarray (24), and PCR FilmArray (25). Other new detection technologies include CHROMagar (26), random amplified polymorphic DNA (27), MALDI-TOF mass spectroscopy (28–30), and PNA-FISH (15, 16, 31, 32). Recently, a new method to detect *Candida* directly from blood samples without culture has been developed, with a limit of detection of 1 CFU/ml of blood and a turnaround time of under 3 h (17, 18).

In our multicenter evaluation of a prototype lot, the *Candida* QuickFISH BC method for rapid identification directly from blood culture bottles of the three most common *Candida* species (*C. albicans*, *C. glabrata*, and *C. parapsilosis*) was found to be sensitive, specific, and rapid. Furthermore, these three species were indeed shown to be the three most common yeast species present in clinical samples, being present in 74.0% of these samples. Individually, *C. glabrata* accounted for 34.5%, *C. albicans* accounted for 30.6%, and *C. parapsilosis* accounted for 8.9% of the clinical isolates. An interesting observation was that *C. glabrata* was the yeast species present in 85.4% (35/41) of the clinical anaerobic bottles studied, a finding in agreement with a recent publication documenting exclusive or earlier growth of *C. glabrata* in anaerobic vials in a large series of candidemic patients from two European hospitals (33).

The *Candida* QuickFISH BC procedure is simple, and results are obtained in about 30 min. All *C. glabrata* (129/129) and *C. albicans* (125/125) isolates and all but one of the 59 *C. parapsilosis* isolates were correctly identified compared to conventional methods. *C. parapsilosis* has recently been reclassified into three species: *Candida parapsilosis* (sensu stricto), *Candida orthopsilosis*, and *Candida metapsilosis* (34). Several *in vitro* studies have demonstrated differences in the geographical distribution, expression of virulence factors, and susceptibility to antifungal agents of these *Candida* spp. (35, 36). Differentiation within the *C. parapsilosis* complex is based on a variety of DNA techniques, such as internal transcriber spacer sequencing (37, 38).

Of the 102 yeast species other than *C. albicans*, *C. glabrata*, and *C. parapsilosis*, the results of *Candida* QuickFISH BC were negative, in agreement with conventional methods, except for two samples, with the two discrepant results associated with false-positive yellow fluorescence for 1 of 5 samples containing *C. metapsilosis* and 1 of 37 samples containing *C. tropicalis*. The overall agreement between *Candida* QuickFISH BC and conventional methods was 99.3% (416/419). The *C. albicans* probe was species

TABLE 4 Comparison between *Candida* QuickFISH BC and conventional identification

Parameter	*C. albicans*	*C. glabrata*	*C. parapsilosis*	Other	Mixed[a]	Total
Conventional *Candida* QuickFISH BC identification (no. of strains)						
C. albicans (green)	125	0	0	0	0	125
C. glabrata (red)	0	129	0	0	0	129
C. parapsilosis (yellow)	0	0	58	2[b]	0	60
Mixed fluorescence	0	0	0	0	4	4
No fluorescence	0	0	1	100	0	101
Agreement, sensitivity, or specificity (%)						
Agreement	100 (125/125)	100 129/129	98.3 (58/59)	98.0 (100/102)	100 (4/4)	99.3 (416/419)
Sensitivity	100 (125/125)	100 (129/129)	98.3 (58/59)	NA[c]	NA	99.7 (312/313)
Specificity	100 (290/290)	100 (286/286)	99.4 (354/356)	99.5 (417/419)	NA	98.0 (100/102)

[a] That is, *C. albicans*/*C. parapsilosis* ($n = 1$), *C. albicans*/*C. glabrata* ($n = 2$), and *C. glabrata*/*C. tropicalis* ($n = 1$).
[b] *Candida metapsilosis* ($n = 1$) and *Candida tropicalis* ($n = 1$).
[c] NA, not applicable.

specific and did not react with any of the other yeast species tested, including 6 *C. dubliniensis* isolates. The *C. parapsilosis* probe did show two false-positive reactions, one with *C. metapsilosis* and the other with *C. tropicalis*, but did not react with 4 *C. orthopsilosis*, 17 other *C. tropicalis*, or 4 other *C. metapsilosis* isolates. The *C. glabrata* probe was species specific, although molecular characterization was not performed to distinguish this species from *C. bracarensis* or *C. nivariensis*.

There are many implications of rapid identification of *C. albicans*, *C. glabrata*, and *C. parapsilosis* on therapy and antimicrobial stewardship. Technologies enabling rapid diagnosis of invasive candidiasis and the determination of the species of *Candida* isolates, such as T2 *Candida*, PNA-FISH, and MALDI-TOF MS, will allow appropriate, cost-effective treatment of patients with candidiasis. This rapid identification of yeast species will limit unnecessary use of antifungal agents and enable antifungal stewardship programs to improve care of patients with systemic candidiasis (39). Antifungal therapy of *Candida* bloodstream infections is frequently inappropriate and delayed, resulting in increased hospital stay and costs (40, 41).

Current clinical practice guidelines of the Infectious Disease Society of America for the management of candidiasis recommend that several factors be considered to guide therapy. These include any history of recent azole exposure and intolerance to antifungal agents, predominant local *Candida* species and susceptibility data, disease severity, the presence of comorbidities, and central nervous system, cardiac valve, or visceral organ involvement (42). These guidelines note that successful treatment depends on early initiation of effective antifungal therapy, with higher mortality rates occurring when therapy is delayed. Fluconazole is recommended as a first-line therapy for patients with mild to moderate illness, who have had no previous azole exposure, and who do not have a high risk of *C. glabrata* infection. Amphotericin B should be used for endocardial or central nervous system infection or an echinocandin for endocardial candidiasis, followed by fluconazole for patients with susceptible isolates who improved clinically after initial therapy. Echinocandins are recommended for patients with moderately severe to severe illness or recent azole exposure, whereas fluconazole is suggested for patients who are not critically ill and have had no recent azole exposure. Echinocandins can be changed to fluconazole for clinically stable patients with *C. albicans* infection. Echinocandins are preferred for infections due to *C. glabrata*, whereas fluconazole is recommended for infections due to *C. parapsilosis*. For *C. krusei* infections, a short course of intravenous echinocandin therapy, followed by oral fluconazole or voriconazole, is suggested.

Limitations of the *Candida* QuickFISH BC assay include the detection of only three yeast species (although this encompasses the most commonly encountered *Candida* species), the relatively high cost of the assay, and the need for well-trained personnel to read the assay using fluorescence microscopy. Another limitation of the study is that a prototype lot of the *Candida* QuickFISH BC assay was used, pending results of stability and functionality of large-scale manufacturing lots, which may require modification of noncoding parts of the probes. Other platforms for the rapid detection of yeasts, such as PCR, are also limited by high cost and a low number of targets, with several assays targeting only a small number of species (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*) due to limitations of the multiplexing capacities of many real-time PCR systems (43–45).

In conclusion, this multicenter study demonstrated the high sensitivity of the Candida QuickFISH BC assay in detecting the three most common *Candida* species associated with bloodstream infections, *C. albicans*, *C. glabrata* and *C. parapsilosis*, and high specificity in differentiating these three *Candida* species from other yeast species.

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