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Low Utility of Pediatric Isolator Blood Culture System for Detection of Fungemia in Children: a 10-Year Review

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The use of the Wampole Isolator 1.5-ml pediatric blood culture tube for the detection of fungemia in children was assessed by a 10-year retrospective review at two pediatric hospitals, The Hospital for Sick Children in Toronto, Canada, and the Children's Medical Center of Dallas, Texas. Over this period, a total of 9,442 pediatric Isolator specimens were processed, with yeast or yeast-like organisms recovered in 297 (3.1%) of the specimens (151 [1.6%] unique clinical episodes) and filamentous or dimorphic fungi recovered in 31 (0.3%) of the specimens (25 unique clinical episodes). Only 18 of the 151 clinical episodes of fungemia attributable to yeast were not detected by automated blood culture systems. The majority of isolated yeast were *Candida* spp., which were usually detected by automated systems, whereas the most common non-*Candida* yeast was *Malassezia furfur*, which the automated system failed to detect. Filamentous or dimorphic fungi were detected in 25 episodes, of which only 9 (36%) episodes were deemed clinically significant after chart review, indicating that in the majority of cases (16/25, 64%) fungal isolation represented contamination. In five of the nine clinically significant episodes, the isolated fungus (*Histoplasma capsulatum*, *Coccidioides immitis/posadasii*, *Fusarium oxysporum*, *Aspergillus* spp., and *Bipolaris* spp.) was also identified in other clinical specimens. Over the 10-year study period, the use of the pediatric Isolator system, at the discretion of the treating physician, only rarely provided useful clinical information for the diagnosis of fungemia in children, with the exception of *M. furfur* and possibly endemic mycoses.

The optimal method for detection of fungi from blood in cases of suspected fungemia or disseminated fungal infection in pediatric patients remains unclear, despite the importance of establishing such a diagnosis, particularly among immunocompromised patients (1, 2). In the past, dedicated systems such as a manual biphasic brain heart infusion (BHI) broth/agar system for the recovery of fungi (including yeasts) from the bloodstream were necessary. Today, however, *Candida* spp., which make up the overwhelming majority of fungal isolates recovered from the blood, are efficiently recovered from automated blood culture systems within a typical 5-day incubation period (3–5). This efficient recovery of *Candida* spp. does, however, rely on multiple cultures since a single blood culture may only detect 65% of episodes of candidemia (6). Much less commonly isolated from automated systems are non-*Candida* fungi, including yeast-like fungi such as *Geotrichum* spp. and *Trichosporon* spp., or certain filamentous fungi such as *Fusarium* spp., *Scedosporium* spp., or *Exophiala* spp., which may represent up to 10% of all fungemias (7).

To overcome the poor recovery of non-*Candida* fungi, other blood culture systems have been developed, such as the Isolator system (Wampole Laboratories, Cranbury, NJ) (8). There is a single published manuscript on the efficacy of the pediatric Isolator for detection of fungemia in children, where it was compared to an early version of the Bactec blood culture system (Bactec NR660; Becton Dickinson and Co., Franklin Lakes, NJ) (9). This study reported on 89 positive fungal blood cultures/4,825 blood culture sets, none of which were filamentous fungi. All fungi isolated were various yeast species, and the investigators found no significant difference between the two blood culture systems for the detection of yeasts.

With the use of the Isolator system, there may also be an increase in the isolation of contaminants compared to automated blood culture systems that may lead to inappropriate treatment (4). Determining the clinical significance of the isolation of a saprophytic filamentous fungus, such as *Aspergillus* or a zygomycete species, is important since these fungi can cause either severe infection or contamination of specimens and/or media (7, 10). Furthermore, it is very uncommon to isolate *Aspergillus* spp. from blood during invasive aspergillosis, despite their overriding importance in disseminated fungal infections. Their recovery usually represents contamination (11).

The Hospital for Sick Children (SickKids) in Toronto, Canada, and the Children's Medical Center (CMC) of Dallas, TX, have utilized the pediatric Isolator 1.5-ml system for the detection of non-*Candida* fungi since it became commercially available. Here, we retrospectively reviewed the yield of the pediatric Isolator system, as used at the discretion of the treating physician, compared to an automated blood culture system, for the detection of all episodes of fungemia over a 10-year period. The clinical signifi-

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TABLE 1 Yeast and yeast-like organisms isolated at SickKids and CMC[a]

Species	SickKids			CMC		
	No. of samples tested using the pediatric Isolator system (n = 2,303)	No. of unique episodes	No. of samples per unique episode	No. of samples tested using the pediatric Isolator system (n = 7,139)	No. of unique episodes	No. of samples per unique episode
Aureobasidium pullulans	0	0	NA	3	1	0
Candida spp.[b]	29	25	2	256	119	11
Ustilago spp.[c]	0	0	NA	3	3	2
Malassezia furfur	6	3	3	0	0	NA
Total	35	28	5	262	123	13

[a] The number of unique episodes refers to the isolation of the same organism from the same patient within 14 days. The number per unique episodes (Isolator only positive) was determined compared to BACTEC or BacT/Alert cultures collected during the same episode. NA, not applicable.
[b] These include seven CMC isolates characterized as "yeast not *Candida albicans*" and one CMC isolate characterized as "yeast isolated."
[c] These include two unique isolates from CMC considered contaminants.

cance of non-*Candida* species isolated from the blood was also assessed through a retrospective clinical review.

MATERIALS AND METHODS

Hospital demographics. SickKids is a 370-bed pediatric hospital, and CMC of Dallas is a 490-bed pediatric hospital system. Services provided at both hospitals include general pediatrics, surgery (general, cardiac, neurosurgery, and orthopedics), cardiac care, general and neonatal intensive care units, oncology and bone marrow transplantation, solid organ transplantation (liver, heart, lung, kidney, and small bowel at SickKids; liver, heart, and kidney at CMC), renal dialysis, burn unit (SickKids only), trauma center, and cystic fibrosis care.

Patient selection. Laboratory data comprising all automated blood cultures processed between 1 July 2003 and 30 June 2013 at SickKids and CMC were analyzed. All patients were 18 years of age or less. At the discretion of the clinical treating team, specimens were also collected in the pediatric Isolator 1.5-ml system for fungal culture, excluding *Malassezia* spp. Culture for *Malassezia* spp. could be ordered upon request at both institutions. Fungal isolates from the pediatric Isolator system and corresponding automated cultures were included in the present study, where the automated cultures were collected ±1 week of collection of the positive Isolator culture, representing a unique episode of infection.

Specimen processing. Routine blood cultures were incubated on the Bactec FX system (Becton Dickinson and Co. [BD], Franklin Lakes, NJ), in BD Bactec Peds Plus/F and BD Bactec Standard/10 Aerobic/F bottles, at SickKids and on the BacT/Alert system (bioMérieux, Laval, Quebec, Canada), in BacT/Alert PF and BacT/Alert FAN bottles, at CMC for a total of 5 days. Positive cultures, with evidence of fungal elements on Gram stain, were planted onto blood, chocolate, MacConkey, CNA (CMC only), Sabouraud, ChromAGAR Candida (BD) (SickKids only, if yeast present), and IMA (CMC only, if evidence of yeast and bacteria). All media were incubated at 35°C in 5% CO_2, with the exception of the MacConkey agar plate at SickKids that was incubated at 35°C in ambient air.

The pediatric Isolator tubes were processed according to manufacturer's recommendations. For fungal blood cultures, the specimen was vortexed and the suspension was planted onto BHI with 5% sheep blood and inhibitory mold agars (IMA), and incubated at 28 to 30°C in O_2. Plates were examined for 21 days (SickKids) and 28 days (CMC). Cultures requested for *Malassezia* spp. were also planted onto IMA with olive oil overlay. Fungal isolates were identified via standard mycological procedures, including macro/microscopic identification and growth conditions, and supplemented with ITS PCR sequencing after 2007 (SickKids) and as needed (CMC) (12).

Clinical significance. The clinical significance of all non-*Candida* fungal species isolated was assessed through a retrospective chart review by an infectious diseases expert, based on the explicit, documented assessment of treating clinicians, the decision to administer a definitive course of antifungal treatment, or a change in patient management occurring as a result of the positive result. In addition, serum galactomannan results were assessed if an *Aspergillus* sp. was isolated.

The study protocol was approved by the research ethics boards at both institutions. The number of colonies recovered in a specimen was also considered. In general, specimens having only one or two fungal colonies were taken to represent contaminants, unless the same organism was also recovered in other cultures (either automated blood cultures or cultures from other sites). All *Candida* isolates were considered clinically significant. Isolation of the same organism from the same patient by the automated system, within 7 days before or after recovery in the Isolator system, was considered to be part of the same clinical episode for the purpose of analysis (i.e., a single, unique episode).

RESULTS

During the 10-year period of the study, a total of 9,442 pediatric Isolator specimens were processed at SickKids and CMC. Yeast or yeast-like organisms were recovered in 297 (3.1%) specimens representing 151 unique episodes (Table 1). This included six *M. furfur* isolates (three episodes) isolated from Isolator tubes plated on IMA with olive oil. Only 13 of the positive Isolator tubes yielded a *Candida* spp. not identified in the automated system, whereas none of the three episodes of *M. furfur* infection were detected by the automated system or the routine Isolator procedure. At both institutions, the majority of isolates were *Candida* spp.

Filamentous or dimorphic fungi were recovered in 31 (0.3%) pediatric Isolator cultures from 25 unique episodes at SickKids (2 isolates, 2 episodes) and CMC (29 isolates, 23 episodes). A chart review revealed that a total of 9 of the 25 episodes (36%) were considered to be clinically significant, while recovery of the isolates during the other 16 episodes were deemed to be due to contamination (Table 2). Serum galactomannan was evaluated in two of the four episodes of aspergillemia, both ~1 week after the collection of the positive blood culture, with both results being negative.

Of the nine clinically significant mold isolates, only *Fusarium oxysporum* was also recovered from the automated blood culture system; this isolate was also recovered from a skin biopsy specimen. None of the other filamentous or dimorphic fungi isolated from the pediatric Isolator system were recovered from the automated blood culture system. The other eight clinically significant isolates included two dimorphic fungi, both isolated at CMC, and six filamentous fungi. The dimorphic fungi, and two of the six

TABLE 2 Filamentous and dimorphic fungi isolated at SickKids and CMC from the pediatric Isolator system compared to corresponding automated system blood cultures

Species	Pediatric Isolator system (n = 9,442)	No. of unique episodes[a]	No. of samples per unique episode			
			Only Isolator blood culture positive[b]	Considered clinically relevant[c]	Recovered in cultures from other sources	Altered appropriate antifungal therapy
Dimorphic fungi						
Histoplasma capsulatum	4	1	1	1	1	0
Coccidioides immitis/posadasii	2	1	1	1	1	0
Subtotal	6	2	2	2	2	0
Filamentous fungi						
Alternaria spp.	2	2	2	0	NA[d]	0
Aspergillus spp.	5	4	4	1	1	0
Bipolaris spp.	2	2	2	1	1	0
Curvularia spp.	2	1	1	1	0	0
Exserohilum spp.	1	1	1	1	0	1
Fusarium oxysporum	1	1	0	1	1	0
Nonsporulating mold	3	3	3	1	0	1
Penicillium spp.	5	5	5	1	0	1
Plectosphaerella cucumerina	1	1	1	0	NA	0
Rhizopus spp.	1	1	1	0	NA	0
Stachybotrys chartarum	1	1	1	0	0	0
Trichoderma spp.	1	1	1	0	NA	0
Subtotal	25	23	22	7	3	3
Total	31	25	24	9	5	3

[a] Isolation of the same organism from the same patient within 14 days.
[b] Compared to BACTEC or BacT/Alert cultures collected during the same episode.
[c] Based on an overall clinical review, as detailed in Materials and Methods.
[d] NA, not applicable.

filamentous fungi, were isolated from other specimens. The two filamentous fungi isolated in other specimens included an *Aspergillus* spp., which was also isolated from a lung biopsy specimen, and a *Bipolaris* spp., which was isolated from pleural fluid (Table 2).

For the patient deemed to have a clinically significant fungemia with an *Aspergillus* spp., the organism was present as a single colony in each of the Isolator tube cultures and the lung biopsy culture, and serum galactomannan testing was negative. In addition to an *Aspergillus* spp., the lung biopsy specimen also grew a single colony of a *Scedosporium* spp. During this clinical episode, six other Isolator cultures were collected and failed to detect any growth. These observations raise the probability that both organisms may have represented contaminants. For our analyses, however, the episode was considered clinically significant because the patient was treated with a course of antifungal medications.

DISCUSSION

The clinical utility of utilizing the pediatric Isolator system, in addition to the use of an automated blood culture system, for the detection of fungemia in children has not been reported to date. We performed a retrospective study on the use of the pediatric Isolator in two exclusively pediatric centers over a 10-year period. Most episodes of candidemia detected by the Isolator system, were also detected by the automated blood culture systems (121/134, 90.3%). In contrast to the high detection rate of *Candida* spp. by both the Isolator and the automated blood culture systems, *M. furfur* was never detected in the automated system, as anticipated. To accurately compare the isolation of *Malassezia* spp. from blood in both systems would require the use of lipid-supplemented media for subculture in each method. This suggests that for the detection of yeasts, the Isolator system provides very little additional microbiologic information, with the exception of the isolation of *M. furfur* due to specific known nutritional requirements.

The additional information gained from the Isolator system, over the automated blood culture system, has been principally examined for adult patients (13–15). Within the adult population, the primary advantage is for the recovery of filamentous and dimorphic fungi, specifically *H. capsulatum* and *C. immitis/posadasii* (15). Within the present study, two episodes of fungemia with dimorphic fungi occurred consisting of a single episode with each of *H. capsulatum* and *C. immitis/posadasii.* In each instance these organisms were not recovered from automated blood cultures. *H. capsulatum* was isolated from the blood of a patient with a positive yeast-phase *Histoplasma* antibody titer (1:8) and a focal liver lesion that yielded *H. capsulatum* on culture. *C. immitis/posadasii* was isolated from a solid-organ transplant patient in whom culture of a bone lesion also yielded the organism. Since these dimorphic fungi were also identified in other, nonblood specimens, the Isolator results did not cause an independent change in treatment.

Four clinically significant mold isolates were recovered from blood using only the Isolator system and were furthermore not recovered from any other body site; these isolates included a *Curvularia* spp., a *Penicillium* spp., an *Exserohilum* spp., and a nonsporulating mold. Since recovery of all of these isolates was considered clinically significant, recovery altered clinical management in each case, with the exception of the *Curvularia* spp.

While the *Curvularia* spp. was isolated from two of four Isolator system specimens, the patient had already been discharged on an empirical course of lipid formulation amphotericin and voriconazole before the cultures became positive, and thus treatment was not subsequently modified.

With only four clinically significant unique episodes of filamentous fungi that were not detected by other means over the course of 10 years (from >9,000 pediatric Isolator specimens processed), the additional benefit of the pediatric Isolator system, based on clinical judgment, appears to be minimal. In addition, it is remarkable that in 16 of 23 (70%) unique episodes, in which a filamentous fungus was recovered from the pediatric Isolator system, the recovered fungus was deemed to be a contaminant. Such results may trigger inappropriate initiation of antifungal therapy prior to the final identification of the isolate. Based on these findings, the use of the pediatric Isolator system has now been discontinued at SickKids. At CMC, targeted education to high-use areas, including the ICU and Hematology/Oncology, was performed; following this educational initiative, utilization of the Isolator system decreased from an average of 949 to 163 the following year. The elimination or reduction of the use of Isolator tubes at our institutions has produced a modest cost savings with respect to the acquisition cost of the tubes themselves but, more significantly, savings in technologist time for processing, and elimination of unnecessary testing and treatment related to false-positive results.

A possible explanation for a low recovery of isolates from the pediatric Isolator system (<4% overall), is the low volume of blood per specimen compared to automated BC bottles which accept a higher volume of blood. Weight-based blood collection guidelines in children for automatic blood cultures may provide a higher probability of recovery than the pediatric Isolator system which utilizes 1.5 ml of blood, which is much less than the recommended sampling volume of 1% total blood volume (16).

This study was limited by the retrospective nature of the design and the limited use of the pediatric Isolator system, which was based solely on the clinical judgment of the treating team. For this reason, and considering the expected disparity between blood volumes inoculated into each system, direct comparisons between the automated and pediatric Isolator systems could not be made in most cases. There were also changes to the volume of blood collected at SickKids in the automated system during the period reviewed, with an emphasis on increased volumes during the later portion of the study. Although this change may have increased the sensitivity of detection of the automated system, and few clinically significant positive specimens were detected by any method throughout the period, this is a limitation of the study. The difference in automated detection methods between the two hospital sites is an additional limitation of the study.

In summary, the addition of the pediatric Isolator system—ordered at the discretion of the treating physician to supplement automated blood culture system isolate recovery—only very rarely provided additional, useful information to the clinician regarding the detection of fungi in children. Specific situations in which the Isolator system may be beneficial are for the detection of *M. furfur* (with the use of lipid-containing subculture media) and endemic mycoses caused by dimorphic fungi such as *H. capsulatum* and *C. immitis/posadasii*. Isolation of these organisms, however, was exceedingly rare in our review of cultures from a 10-year period, and the majority of organisms recovered are *Candida* spp., which are routinely recovered from automated blood culture systems. Furthermore, the majority of filamentous fungi recovered from the Isolator system were not deemed clinically significant, as determined through a retrospective clinical analysis.

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