JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1996, p. 1877–1879 Vol. 34, No. 8
0095-1137/96/$04.00+0

Comparison of Isolator 1.5 and BACTEC NR660 Aerobic 6A Blood Culture Systems for Detection of Fungemia in Children

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Received 8 March 1996/Returned for modification 11 April 1996/Accepted 4 May 1996

The Isolator 1.5 microbial system (ISO 1.5) (Wampole Laboratories, Cranbury, N.J.) was compared with the BACTEC NR660 aerobic NR6A bottle (NR6A) (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) for the detection of fungemia in hospitalized pediatric patients. For 4,825 paired blood cultures evaluated retrospectively from April 1992 to December 1994, at least one blood culture system was positive for 89 clinically important fungal isolates involved in 36 episodes of fungemia in 34 patients. Sixty isolates (44 *Candida albicans*, 12 *Candida parapsilosis*, and 4 *Candida tropicalis* isolates) were recovered from both systems, 13 were recovered from NR6A bottles only (10 *C. albicans*, 1 *C. parapsilosis*, and 2 *Cryptococcus neoformans* isolates), and 16 were recovered from ISO 1.5 tubes only (8 *C. albicans* and 5 *C. parapsilosis* isolates and 1 *C. tropicalis*, 1 *Candida lusitaniae*, and 1 *Rhodotorula glutinis* isolate) ($P > 0.05$). For the 60 *Candida* isolates from both systems, the mean time to detection was the same in each system. Thirty-seven isolates were detected by both systems on the same day, 9 isolates were detected earlier by NR6A, and 14 isolates were detected earlier by ISO 1.5 ($P > 0.05$). Of the 36 clinically important episodes of fungemia, 22 were detected by both systems (13 *C. albicans* isolates and 9 other *Candida* isolates), 4 were detected by NR6A only (3 *C. albicans* isolates and 1 *C. neoformans* isolate), and 10 were detected by ISO 1.5 only (3 *C. albicans* isolates, 6 other *Candida* isolates, and 1 *R. glutinis* isolate) ($P > 0.05$). Of the 22 episodes in which cultures from both systems were positive at some point during the episode, 12 were detected on the same day by both systems, 8 were detected earlier by NR6A, and 2 were detected earlier by ISO 1.5. Thus, for our pediatric population, NR6A is comparable to ISO 1.5 in both yield and time to detection of yeasts in fungemic patients.

Over the past decade, hospital-acquired bloodstream infections with fungi in pediatric patients have become increasingly important (13). In some tertiary-care centers, fungemia accounts for up to 10% of all cases of sepsis in children (11, 12). Although specific uses of fungal blood culture methods have been recommended for adults (19), there are no established guidelines for the laboratory diagnosis of fungemia in pediatric practice. This issue is of particular importance for children because of the minimal volumes of blood obtained for culture. Since Isolator 1.5 microbial tubes have been used routinely in our hospital for the recovery of fungi from children, we had the opportunity to assess retrospectively the incremental utility of the lysis-centrifugation method versus BACTEC NR6A broth medium for the isolation of yeasts and other fungi.

(This study was presented in part at the 95th General Meeting of the American Society for Microbiology, Washington, D.C., on 21 May 1995 [11a].)

MATERIALS AND METHODS

Patient selection. Microbiology laboratory reports of blood cultures received from inpatient pediatric wards from April 1992 to December 1994 at Duke University Medical Center, Durham, N.C., were reviewed retrospectively. Only patients having one or more paired blood cultures were included in the analysis. Paired blood cultures were defined as one BACTEC nonradiometric 660 aerobic NR6A bottle (NR6A) (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) and one Isolator 1.5 microbial tube (ISO 1.5) (Wampole Laboratories, Cranbury, N.J.) received in the laboratory on the same day. Positive cultures were defined as paired blood cultures with at least one system positive.

Blood culture processing. The NR6A bottles were processed on BACTEC 660 blood culture instruments and were incubated for 7 days at 35°C with shaking for the first 48 h. Bottles were tested for growth of microorganisms once on the day of receipt, twice on day 2, and once daily on days 3 through 7. When yeasts were detected in positive bottles by Gram stain, India ink preparation, or both, chocolate agar and inhibitory mold agar were inoculated for aerobic subculture and incubated at 37°C in 7% CO_2 for examination at 24 and 48 h. The time to detection for NR6A was recorded as the time at which the Gram stain was positive.

All ISO 1.5 tubes were processed within 8 h of collection. The centrifuged lysate was mixed vigorously for 10 s, and equal aliquots were inoculated onto chocolate agar and brain heart infusion agar or inhibitory mold agar with gentamicin as well as brain heart infusion agar with 10% sheep blood. For patients less than 9 months old or those receiving total parenteral nutrition, 3 drops of sterile olive oil were added to the brain heart infusion agar blood plate. All plates were incubated at 37°C in 7% CO_2, examined daily for the first 7 days, and held until 14 days before final readings. The time at which fungal growth was detected macroscopically on agar plates was recorded as the time to detection for the Isolator system.

If growth was detected macroscopically either on ISO 1.5 plates or in NR6A subcultures suspicious for yeasts, a germ tube test was performed. All yeasts with a positive test were reported as *Candida albicans* (16). All molds and yeasts with a negative germ tube test were processed according to standard mycological procedures for further identification (16). Yeasts with a negative germ tube test were identified by carbohydrate fermentation reactions, rapid urease tests, morphology on cornmeal agar, and, when necessary, carbohydrate assimilation tests (API 20C; bioMérieux-API, St. Louis, Mo.).

Clinical assessment. The clinical significance of microorganisms isolated from blood cultures was determined by either physician evaluation or review of the patient's medical record. An episode of fungemia was defined as beginning with the first positive blood culture recovered from a patient. A second episode was defined by negative cultures for 48 h followed by a positive culture or the presence of a new isolate. The speed of recovery for each fungal episode was evaluated for those episodes in which paired blood cultures were positive in both systems at some point during the episode.

Data analysis. All comparisons were evaluated statistically by the McNemar modification of the chi-square test (10).

RESULTS

During the study period, a total of 14,214 NR6A and 5,067 ISO 1.5 cultures were received. Fungi grew in 149 (1.1%)

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TABLE 1. Comparative yields of clinically important fungi recovered from NR6A bottles and ISO 1.5 tubes

Yeast	No. of isolates recovered from:		
	Both systems	NR6A bottles only	ISO 1.5 tubes only
C. albicans	44	10	8
C. parapsilosis	12	1	5
C. tropicalis	4	0	1
C. lusitaniae	0	0	1
C. neoformans	0	2	0
R. glutinis	0	0	1
Total	60	13	16[a]

[a] *P* value is not statistically significant ($P > 0.05$).

NR6A bottles and were recovered from 102 (2.0%) ISO 1.5 tubes. A total of 4,825 cultures were paired and fulfilled the study criteria. At least one blood culture system was positive for a fungus in 91 (1.9%) paired cultures. Eighty-nine of these 91 paired cultures were clinically important and were involved in 36 episodes of fungemia in 34 patients. There were two repeated episodes of fungemia. Of the 89 positive cultures, 87 were pure cultures, 1 NR6A culture was *C. albicans* mixed with an enterococcus, and 1 NR6A culture was *Candida parapsilosis* mixed with coagulase-negative staphylococci. Single colonies of molds recovered only from ISO 1.5 tubes in two paired cultures were excluded from the study, because they were judged to be contaminants by clinical criteria. *Malassezia furfur* was recovered from a single patient once from an ISO 1.5 culture and once from an NR6A culture but not in cultures received on the same day.

Sixty (67%) clinically important fungal isolates were recovered from both systems, 13 (15%) were recovered from NR6A bottles only, and 16 (18%) were recovered from ISO 1.5 tubes only (Table 1). Of the 60 *Candida* isolates from both systems, 37 were detected by both systems on the same day, 9 were detected earlier (≥24 h) in NR6A bottles, and 14 were detected earlier in ISO 1.5 tubes ($P > 0.05$). The mean times to detection (excluding 2 *C. albicans* isolates detected on day 13 in ISO 1.5 tubes) were 2.1 days in NR6A and 1.9 days in ISO 1.5.

Of the 36 clinically important episodes of fungemia, 22 (61%) were detected by both systems, 4 (11%) were detected by NR6A only, and 10 (28%) were detected by ISO 1.5 only (Table 2). Of the 22 episodes in which both systems were positive at some point during the episode, 12 were detected on the same day by both systems, 8 were detected earlier by NR6A, and 2 were detected earlier by ISO 1.5 ($P > 0.05$).

TABLE 2. Comparative yields by episode of fungi recovered from NR6A bottles and ISO 1.5 tubes

Yeast	No. of episodes detected by:		
	Both systems	NR6A bottles only	ISO 1.5 tubes only
C. albicans	13	3	3
C. parapsilosis	7	0	4
C. tropicalis	2	0	1
C. lusitaniae	0	0	1
C. neoformans	0	1	0
R. glutinis	0	0	1
Total	22	4	10[a]

[a] *P* value is not statistically significant ($P > 0.05$).

DISCUSSION

Although several authors have compared the ISO 1.5 and broth blood culture systems, currently there are no reports of studies that have assessed the applied utility of ISO 1.5 in the detection of fungemia (4, 5, 7, 11, 12, 14, 17) in children. In our study, NR6A was equivalent to ISO 1.5 in both yield and time to detection of yeasts in fungemic patients. Overall, there was no statistically significant advantage of either system for the recovery of *Candida* spp. by either specimen or episode. Six of nine episodes with *Candida* spp. detected only by ISO 1.5 had only a single culture that was positive when multiple blood cultures had been obtained, including all four episodes with *C. parapsilosis*. There were three episodes with *Candida* spp. detected by NR6A only, of which two had single cultures that were positive when multiple blood cultures had been obtained. The clinical importance of single blood cultures positive for yeasts is difficult to determine, particularly in this study setting, where many of the patients were immunocompromised because of chemotherapy, bone marrow transplantation, or underlying hematological diseases and some were already receiving antifungal therapy (13). All eight of the single positive blood culture isolates were considered clinically important, and the physician thresholds for both major and minor clinical interventions were low, especially in six episodes that occurred in bone marrow transplant recipients. Hence, the clinical importance of a single positive fungal blood culture when multiple blood cultures have been obtained remains equivocal.

Although no extensive data are available for ISO 1.5 in the detection of fungemia in children, the Isolator 10-ml microbial tube has been reported as having advantages for adults in the recovery of non-*Candida* yeasts and molds, particularly *Cryptococcus neoformans* and *Histoplasma capsulatum* (1–3, 8, 15, 18). In our study, ISO 1.5 recovered the only isolate of *Rhodotorula glutinis*. The two molds recovered only by ISO 1.5 were deemed plate contaminants; each was present as a single colony of mold and resulted in no change in patient management. NR6A recovered the only two isolates of *C. neoformans*, and the companion ISO 1.5 tubes failed to recover this microorganism. As expected, *H. capsulatum* was not recovered during the study period, since our patients come mostly from areas of nonendemicity.

Since our study was a retrospective analysis and focused on children from whom only small samples of blood often were obtained, the volume of blood inoculated into the NR6A bottles was variable and may have ranged from 1 to 5 ml compared with the 1 to 1.5 ml inoculated into ISO 1.5 tubes. Hence, NR6A may have had a volume advantage, but at our institution most pediatric blood culture bottles are inoculated with less than 2 ml of blood. Another possible limitation in this retrospective analysis was defining paired blood cultures as those received in the laboratory on the same day. Nonetheless, a recent study on the detection of bacteremia has shown that no significant difference in yield was obtained whether cultures were drawn simultaneously or 24 h apart (9).

Although broth-based procedures do not allow quantitation, the BACTEC system has low contamination rates (4, 5) and, according to College of American Pathologists workload values (6), BACTEC 660 requires 4.3 min per bottle for processing. The lysis-centrifugation system has higher contamination rates (4, 5, 7), and is more labor-intensive, requiring 8.0 min per ISO 1.5 tube for processing. If ISO 1.5 is frequently used in

addition to broth cultures for the sole purpose of recovering fungi, it could increase health care costs substantially in populations such as our study population. The additional patient charges for the extra 4,825 companion culture ISO 1.5 tubes that were coupled with NR6A cultures over 18 months in this study were $294,325, and the estimated labor and supply costs were approximately $45,000.

In summary, NR6A was equivalent in practice to ISO 1.5 in both yield and time to detection of yeasts, particularly *Candida* spp. Since obtaining adequate volumes of blood in the pediatric population is often difficult, the selection of a sensitive blood culture system is of paramount importance. In this retrospective study, BACTEC NR6A was a sensitive and rapid method for detecting fungemia in the initial evaluation of sepsis in children. Consequently, we now rely on broth cultures for the routine initial evaluation of suspected sepsis with bacteria and yeasts. ISO 1.5 tubes now are used only in consultation with the Clinical Microbiology Laboratory and infectious-disease specialists as a supplemental procedure, e.g., for referred patients in whom infection with *H. capsulatum* is suspected on the basis of clinical findings and geographical exposure.

ACKNOWLEDGMENT

This work was supported in part by an Aga Khan Foundation scholarship award (to A. K. M. Zaidi).

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