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Comparison of BACTEC MYCO/F LYTIC and WAMPOLE ISOLATOR 10 (Lysis-Centrifugation) Systems for Detection of Bacteremia, Mycobacteremia, and Fungemia in a Developing Country

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In less-developed countries, studies of bloodstream infections (BSI) have been hindered because of the difficulty and costs of culturing blood for bacteria, mycobacteria, and fungi. During two study periods (study period I [1997] and study period II [1998]), we cultured blood from patients in Malawi by using the BACTEC MYCO/F LYTIC (MFL), ISOLATOR 10 (Isolator), Septi-Chek AFB (SC-AFB), and Septi-Chek bacterial (SC-B) systems. During study period I, blood was inoculated at 5 ml into an MFL bottle, 10 ml into an Isolator tube for lysis and centrifugation, and 10 ml into an SC-B bottle. Next, 0.5-ml aliquots of Isolator concentrate were inoculated into an SC-AFB bottle and onto Middlebrook 7H11 agar slants, chocolate agar slants, and Inhibitory Mold Agar (IMA) slants. During study period II, the SC-B and chocolate agar cultures were discontinued. MFL growth was detected by fluorescence caused by shining UV light ($\lambda = 365$ nm) onto the indicator on the bottom of the bottle. During study period I, 251 blood cultures yielded 44 bacterial isolates. For bacteremia, the MFL was similar to the Isolator concentrate on chocolate agar (34 of 44 versus 27 of 44; P, not significant [NS]), but more sensitive than the SC-B bottle (34 of 44 versus 24 of 44; $P = 0.05$). For both study periods combined, 486 blood cultures yielded 37 mycobacterial and 13 fungal isolates. For mycobacteremia, the sensitivities of the MFL and Isolator concentrate in the SC-AFB bottle were similar (30 of 37 versus 29 of 37; P, NS); the MFL bottle was more sensitive than the concentrate on Middlebrook agar (30 of 37 versus 15 of 37; $P = 0.002$). For fungemia, the MFL bottle was as sensitive as the SC-B bottle or Isolator concentrate on chocolate agar or IMA slants. We conclude that the MFL bottle, inoculated with just 5 ml of blood and examined under UV light, provides a sensitive and uncomplicated method for comprehensive detection of BSI in less-developed countries.

The study of bloodstream infections (BSI) in less-developed countries requires culturing of blood for bacteria, mycobacteria, and fungi (1, 2, 9) and is made particularly difficult for the following reasons: (i) inadequate microbiology resources or laboratory capacity for processing blood cultures; (ii) lack of trained personnel or inadequate training of personnel; (iii) prohibitive costs for the services of skilled personnel, materials, or laboratory equipment; and (iv) extensive technician time required to process multiple media and stages (including quality control) to identify the various pathogens.

In studies in Tanzania and Thailand, countries with high prevalences of human immunodeficiency virus type 1 (HIV-1), we cultured blood for bacteria, mycobacteria, and fungi by using three blood culture systems simultaneously; (i) the Septi-Chek (Becton Dickinson Microbiology Systems [BDMS], Cockeysville, Md.) biphasic bacterial blood culture bottle (SC-B), (ii) the ISOLATOR 10 (Isolator) lysis-centrifugation system (Wampole Laboratories, Cranbury, N.J.), and (iii) the Septi-Chek AFB (BDMS) biphasic mycobacterial blood culture bottle (SC-AFB) containing Middlebrook 7H9 broth (1, 2). These three blood culture systems, although sensitive for recovery of bacteria, fungi, and mycobacteria, required 20 to 25 ml of blood and considerable time, effort, and resources for processing.

Recently, the BACTEC MYCO/F LYTIC (MFL; BDMS) blood culture bottle was introduced and licensed for use in the United States to detect mycobacteremia (10). Each MFL bottle contains 40 ml of nonselective culture medium (supplemented Middlebrook 7H9 and brain heart infusion broth), specific proteins and sugars, saponin (a blood lysing agent), and added oxygen and carbon dioxide. At the bottom of each vial, there is a sensor that can detect decreases in oxygen concentration resulting from growth of microorganisms. Growth of microorganisms may be detected in the automated BACTEC 9000 series systems or manually by shining the UV light of a Wood's lamp on the sensor at the bottom of the bottle; the resulting fluorescence is growth dependent and is proportional to the decrease in oxygen in the vial.

We conducted this study to determine the etiology of BSI in a febrile, adult population in Malawi and to compare the MFL blood culture system with the SC-B, SC-AFB, and Isolator systems for yield of microorganisms.

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MATERIALS AND METHODS

Patients. During August to October 1997 (study period I) and March to May 1998 (study period II), consecutive febrile, adult patients who were admitted to the Lilongwe Central Hospital (LCH), Lilongwe, Malawi, were enrolled in this study after giving informed consent. The study protocol was reviewed and approved by institutional review boards at the Centers for Disease Control and Prevention and at the Ministry of Health, Lilongwe, Malawi. Malawi is a developing country in sub-Saharan Africa; LCH is a 300-bed district general hospital located in Lilongwe.

Blood culture collection. After the patient's skin had been cleaned with a 2% tincture of iodine and 70% alcohol, venous blood was drawn with a needle and syringe in a single venisection and aseptically inoculated into the various blood culture systems. During study period I, 25 ml of blood was inoculated as follows: 5 ml into an MFL bottle, 10 ml into an Isolator tube, and 10 ml into an SC-B blood culture bottle. During study period II, inoculation of blood into the SC-B bottle was discontinued. Study patients did not have repeat blood cultures.

Processing of samples. In the laboratory, the blood in the Isolator tube underwent lysis and centrifugation within 8 h of venisection, according to the manufacturer's directions. A 0.5-ml aliquot of the lysis-centrifugation concentrate was inoculated into an SC-AFB bottle containing Middlebrook 7H9 broth to which Acid Fast Bacilli Culture Supplement (BDMS) was added and to which an AFB agar paddle (BDMS) was attached in the laboratory. Also, 0.5-ml aliquots of the Isolator concentrate were inoculated onto Middlebrook 7H11 agar slants, heated blood (chocolate) agar slants, and Inhibitory Mold Agar (IMA) slants (BDMS). An agar slide paddle (BDMS) was attached to the SC-B bacterial blood culture bottle, which then was momentarily inverted so that the contents covered the agar paddle. All blood culture bottles and agar slants were incubated aerobically at 35°C. Chocolate agar slants were incubated in a 5% carbon dioxide atmosphere. Inoculation of the Isolator concentrate onto chocolate agar slants too was discontinued during study period II.

Chocolate agar and IMA slants were read macroscopically daily during the first week and then weekly for 4 weeks or until growth was observed; Middlebrook 7H11 slants were read once weekly for 8 weeks or until growth was observed. The MFL blood culture bottles were read for evidence of growth by using a handheld UV (λ = 365 nm) lamp to detect the fluorescence of the indicator at the bottom of the bottle; SC-B bottles were read macroscopically. All MFL and SC-B bottles were examined for signs of growth twice in the first 24 h following incubation and then daily for the next 7 days. Broth from SC-B bottles that remained clear after 7 days was terminally subcultured onto sheep blood agar plates. SC-AFB bottles were inverted and rotated daily to cover the agar paddle during the first week and then once weekly for 8 weeks or until growth was observed. Preliminary identification of bacteria and fungi was conducted on-site by using standard microbiologic tests.

All blood culture bottles and all bacteria, fungi, or mycobacterial organisms that were isolated at the study site during the two study periods were transported to the Clinical Microbiology Laboratory at Duke University Medical Center, where mycobacterial and fungal cultures were processed and the identities of bacterial and fungal isolates were confirmed. *Mycobacterium* spp. were identified by using DNA probes (AccuPROBE; Gen-Probe, San Diego, Calif.) for *Mycobacterium tuberculosis* complex and *Mycobacterium avium* complex, biochemical tests, and high-performance liquid chromatography.

Clinical assessment. Each positive blood culture for bacteria or fungi was reviewed by one of the principal investigators at the study site. Isolates such as *Bacillus* spp., coagulase-negative *Staphylococcus* spp., diphtheroids, or *Micrococcus* spp. were deemed contaminants (11).

Data analysis. The MFL system was compared with the other blood culture systems for detection of bacteremia, mycobacteremia, and fungemia by using the McNemar modification of the chi-square test and, where appropriate, the Yates correction for small numbers of observations.

RESULTS

Two hundred fifty-one single blood cultures were drawn during study period I, and 235 were drawn during study period II. Thus, a total of 486 single blood cultures were obtained from adult patients admitted to LCH with fever during both study periods combined. Non-serovar Typhi *Salmonella enterica*, *Streptococcus pneumoniae*, and *M. tuberculosis* were the most common BSI isolates. For study periods I and II combined, a total of 8 (1.6%) blood cultures yielded organisms that were considered contaminants, including coagulase-negative *Staphylococcus* spp., diphtheroids, and *Micrococcus* spp. Four of these contaminants occurred at inoculation of an MFL or SC-B bottle; the remainder grew only on the Middlebrook 7H11 agar. Thus, the contamination of blood culture bottles occurred in just 4 (0.8%) of the 486 blood cultures obtained.

TABLE 1. Bacterial pathogens isolated from blood cultures, Lilongwe Central Hospital, Malawi, during study period I (1997)

Pathogens	No. of bacterial isolates recovered by:			
	All systems	MFL bottle	SC-B bottle	Isolator concentrate on chocolate agar
Streptococcus pneumoniae	24	19	17	20
Salmonella strains[a]	13	9	4	5
Enterobacteriaceae[b]	5	5	2	1
Other gram-positive cocci[c]	2	1	1	1

[a] Eight *Salmonella enterica* serovar Typhimurium, three *S. enterica* serovar Enteritidis, and two *S. enterica* serovar Typhi isolates.
[b] Three *Escherichia coli* and two *Klebsiella* isolates.
[c] One *Streptococcus pyogenes* isolate and one *Streptococcus sanguis* isolate.

Detection of bacteremia (study period I). During study period I, we assessed the growth of bacteria in 251 blood cultures by using three blood culture systems (MFL, SC-B bottle, and Isolator concentrate on chocolate agar). From these 251 blood cultures, 44 bacterial pathogens grew in one or more of the three systems (Table 1). Overall, the MFL system had a bacterial recovery rate that was similar to that of the Isolator concentrate inoculated on chocolate agar (34 of 44 versus 27 of 44; *P*, NS). However, the MFL bottle had a higher bacterial recovery rate than the SC-B bottle (34 of 44 versus 24 of 44; *P* < 0.05).

Detection of bacteremia (study period II). During study period II, only the MFL bottle was used to detect bacteremia. We did not continue with the SC-B bottle or inoculation of the Isolator concentrate on chocolate agar, because their relatively lower diagnostic yield for bacteria did not warrant collection of an extra 10 ml of blood for the former or the extra work involved in the latter. Of the 235 MFL bottles inoculated during this period, 45 (19%) yielded bacterial growth.

Detection of mycobacteremia (study periods I and II combined). We assessed the growth of mycobacteria in 486 cultures in each of three systems (MFL, the Isolator concentrate inoculated into the SC-AFB bottle, and the Isolator concentrate on Middlebrook 7H11 agar). From these 486 blood cultures, 37 mycobacterial species were detected by one or more of the three systems. The MFL bottle and the Isolator concentrate in the SC-AFB bottle had similar rates of detection of mycobacteria (30 of 37 versus 29 of 37; *P*, NS). However, the MFL bottle had a higher rate of isolation of mycobacteria than did the Isolator concentrate on Middlebrook 7H11 agar slants (30 of 37 versus 15 of 37; *P* = 0.002) (Table 2).

Detection of fungemia (study period I). The MFL system had a higher fungal recovery rate than did the SC-B bottle (3 of 5 versus 0 of 5; *P*, NS) or the Isolator concentrate on chocolate agar (3 of 5 versus 1 of 5; *P*, NS); this difference did not reach statistical significance.

Detection of fungemia (study periods I and II combined). The MFL system had a lower fungal recovery rate than the Isolator concentrate on IMA (6 of 13 versus 10 of 13; *P*, NS); this difference, however, did not reach statistical significance.

Time of recovery (bacterial and fungal isolates). During study period I, the reading intervals of the SC-B, MFL, and Isolator concentrate-on-chocolate agar systems for the detection of bacteremia during the first week were similar. The overall median times to detect bacteria and fungi were 1 day (range, 1 to 4) for the MFL system, 1 day (range, 1 to 3) for the SC-B system, and 2 days (range, 1 to 3) for the Isolator concentrate on chocolate agar. The median time for detecting

TABLE 2. Mycobacterial species isolated from blood cultures, Lilongwe Central Hospital, Malawi, during study periods I (1997) and II (1998)

Pathogens	No. of mycobacterial isolates recovered by:			
	All systems	MFL bottle	Isolator concentrate in SC-AFB bottle	Isolator concentrate on Middlebrook 7H11 agar
Mycobacterium tuberculosis	33	27	25	13
Mycobacterium simiae or SAV[a]	2	2	2	1
Mycobacterium bovis	1	0	1	0
Mycobacterium avium complex	1	1	1	1

[a] Mycobacterial species of the SAV group resemble *M. avium* complex by conventional biochemical tests, including a negative niacin reaction (true *M. simiae* strains have positive niacin reactions), but have high-performance liquid chromatography profiles that are consistent with *M. simiae*.

fungi by culturing the Isolator concentrate on IMA was 3.5 days (range, 3 to 10).

Time of recovery (mycobacterial isolates). Although the reading intervals for mycobacteremia in MFL, SC-AFB, and Middlebrook 7H11 agar cultures were similar during the initial microbiology workup in Malawi, we were unable to determine growth during shipment of these cultures to the United States and therefore were unable to assess relative times of detection.

DISCUSSION

Previous studies of BSI in regions of the world with high HIV-1 endemicity have established that mycobacteria, bacteria, and fungi are all important causes of BSI and should be comprehensively sought (1, 2, 9). Although the methodologies in these three studies were sensitive for recovery of the three classes of pathogens, the volumes of blood required for comprehensive culture were high (20 to 30 ml), and processing of the Isolator tubes followed by inoculation of the concentrate into Middlebrook broth and onto solid media required additional laboratory steps that are not otherwise necessary during inoculation and processing of blood culture bottles. These additional steps required multiple kinds of culture media and additional laboratory technician time. Moreover, the risk of contamination with environmental organisms is increased by using the Isolator tube and lysis-centrifugation system (4–6). In developing countries with limited resources, such added costs are not sustainable. In addition, where clinical microbiology facilities exist for processing mycobacterial blood cultures, testing every patient admitted to hospital may be costly or may require inappropriate use of already scarce microbiology resources. Other factors that preclude the processing of mycobacterial blood cultures in these laboratories include the need for a dedicated carbon dioxide incubator and limited refrigerated facilities for the storage of several kinds of media.

Previous comparative studies have demonstrated that the SC-B system is able to detect more bacterial microorganisms associated with sepsis than conventional broth cultures (12) and a similar number of bacterial pathogens to the BACTEC radiometric system (13). Lysis-centrifugation using the Isolator tube has been shown to detect microbiologically proven bacteremias or fungemias in significantly greater numbers than either conventional tryptic soy broth bottles with CO_2 or a biphasic bacterial blood culture bottle (7). Direct inoculation of Middlebrook 7H11 conventional solid media and direct inoculation of broth media with Isolator concentrate remain two of the most widely used methods for recovery of mycobacteria from blood (3, 8). In our study, we inoculated Isolator concentrate into the SC-AFB biphasic bottle, which contains Middlebrook 7H9 broth. This method was previously used to culture blood for mycobacteria in Tanzania and Thailand (1, 2) and was found to be more sensitive for recovery of mycobacteria than inoculating the Isolator pellet onto solid Middlebrook 7H11 agar (unpublished data).

For any of the methods presented above, culturing blood comprehensively for bacteria, mycobacteria, and fungi requires drawing at least 20 to 30 ml of blood from adult patients. For cultural reasons, patients in various countries are loathe to part with seemingly large volumes of blood. The data from our study indicate that the MFL bottle inoculated with just 5 ml of blood is (i) superior to the SC-B bottle inoculated with 10 ml of blood and similar to lysis-centrifugation for detection of bacteremia and fungemia and is (ii) superior or similar to Isolator concentrate inoculated on solid media or in Middlebrook broth, respectively, for detection of mycobacteria.

The present study was conducted in a region in Malawi where nearly three-quarters of febrile adults presenting to the regional general hospital are HIV-1 infected (L. C. McDonald, L. K. Archibald, O. Nwanyanwu, P. Kazembe, L. B. Reller, and W. R. Jarvis, 47th Annu. Epidemic Intell. Service (EIS) Conf., oral presentation, 1998). During processing, the Isolator-lysis-centrifugation system requires several manipulations that potentially could result in needle stick injury, cuts from broken Isolator tubes, or aerosol exposure to Isolator tube contents. Thus, for laboratory personnel in regions of HIV-1 endemicity, use of the Isolator system increases the risk of exposure to the HIV-1 virus.

For less-developed world settings, where blood cultures either are or should be part of the microbiology service repertoire, the MFL blood culture system has several advantages: one bottle will yield growth of bacteria, mycobacteria, or fungi; it does not require a carbon dioxide incubator; it is compact and easily stored; and it is easily read by manually shining a UV light on the bottom of the bottle. Our study demonstrates that comprehensive culture for the three classes of pathogens could now be achieved by inoculating just 5 ml of blood into one blood culture bottle rather than inoculating aliquots of blood into various bottles, tubes, or culture media. The ease of a single inoculation into one blood culture bottle circumvents the necessity of multiple inoculations, processing steps, and subculturing onto various culture media, thereby reducing rates of contamination, the amount of technician time required for processing, and the risk of needle stick injuries.

The rate of blood culture contamination in this study was low (1.6% overall and <1% when contamination of Middlebrook 7H11 agar slants was excluded). These low rates were achieved by ensuring that high aseptic standards were maintained before and during venipuncture, using both alcohol and iodine for skin cleansing and allowing the skin to dry properly before venisection, as well as scrupulous cleaning of the rubber diaphragms of blood culture bottles and Isolator tubes with isopropyl alcohol before inoculation with blood and before

accessing the needle and syringe during microbiologic processing of blood culture bottles and Isolator tubes.

In the United States, the MFL bottle with the BACTEC 9000 series automated machines is marketed as a system for the growth and detection of mycobacteria. Because the MFL blood culture medium is not selective and will support the growth of aerobic organisms, such as mycobacteria, bacteria, yeasts, and fungi, the presence of other organisms in the blood may inhibit growth of mycobacteria in it. While this might constitute a limitation in the United States, it is this property that renders the MFL medium, in combination with a handheld UV lamp, an excellent system for the comprehensive detection of mycobacteremia, bacteremia, or fungemia in developing countries.

There were three limitations to our study. (i) While the MFL blood culture system was as sensitive as the Isolator concentrate inoculated on IMA in detecting fungi, the numbers of these isolates (11 *C. neoformans* isolates, 1 *Cryptococcus laurentii* isolate, and 1 *Candida tropicalis* isolate) were too small to enable a valid assessment of its fungal detection capability. For similar reasons, Waite and Woods also were not able to make any general inferences about the reliability of the MFL bottle for the recovery of fungi (10). (ii) The predominance of one organism may interfere with the recovery of other types and therefore prevent the detection of polymicrobial BSI. (iii) Because the MFL and SC-AFB bottles and the Middlebrook 7H11 agar slants were shipped to the United States for further processing, we were unable to assess and compare relative times of detection of mycobacteria by these respective recovery methods. As expected for most comparative studies of this type, there was a bias with regard to the volume of blood inoculated into the various media. Ten milliliters of blood each was inoculated into the SC-B bottle and Isolator tube; 5 ml was inoculated into the MFL bottle as recommended by the manufacturer. Thus, it is plausible that the recovery rate of microorganisms from the MFL system could have been higher if the inoculation volumes of blood in MFL bottles were >5 ml.

In conclusion, one MFL blood culture bottle, inoculated with just 5 ml of blood, was comparable to the SC-B, SC-AFB, or Isolator blood culture systems in detecting bacteremia, mycobacteremia, and fungemia in a febrile adult population in a region of HIV-1 endemicity. Moreover, growth in an MFL bottle was easily detected by shining a UV lamp on the indicator located in the base of the bottle. The MFL system provides a sensitive and uncomplicated method for comprehensive detection of BSI in developing countries.

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