JCM
Journals.ASM.org

Contribution of the BacT/Alert MB *Mycobacterium* Bottle to Bloodstream Infection Surveillance in Thailand: Added Yield for *Burkholderia pseudomallei*

Possawat Jorakate,[a] Melissa Higdon,[a*] Anek Kaewpan,[a] Sirirat Makprasert,[a] Somkhit Yuenprakhon,[c] Kittisak Tawisaid,[d] Surang Dejsirilert,[e] Toni Whistler,[a,b] Henry C. Baggett[a,b]

Global Disease Detection, Thailand Ministry of Public Health-U.S. Centers for Disease Control and Prevention (CDC) Collaboration, Nonthaburi, Thailand[a]; Division of Global Health Protection, CDC, Atlanta, Georgia, USA[b]; Sa Kaeo Crown Prince Hospital, Sa Kaeo, Thailand[c]; Nakhon Phanom Provincial Hospital, Nakhon Phanom, Thailand[d]; National Institute of Health, Ministry of Public Health, Nonthaburi, Thailand[e]

Community-acquired bloodstream infections cause substantial morbidity and mortality worldwide, but microbiology capacity and surveillance limitations have challenged good descriptions of pathogen distribution in many regions, including Southeast Asia. Active surveillance for bloodstream infections has been conducted in two rural Thailand provinces for >7 years. Blood specimens were divided into two culture bottles, one optimized for aerobic growth (F bottle) and a second for enhanced growth of mycobacteria (MB bottle), and processed with the BactT/Alert 3D system. Because the routine use of MB culture bottles is resource intensive (expensive and requires prolonged incubation), we assessed the added yield of MB bottles by comparing the proportion of pathogens detected by MB versus that by F bottles from 2005 to 2012. Of 63,066 blood cultures, 7,296 (12%) were positive for at least one pathogen; the most common pathogens were *Escherichia coli* (28%), *Burkholderia pseudomallei* (11%), *Klebsiella pneumoniae* (9%), and *Staphylococcus aureus* (6%). Two bottles improved the yield overall, but the added yield attributable to the MB bottles was limited to a few pathogens. In addition to the detection of mycobacteria and some fungi, MB bottles improved the detection of *B. pseudomallei* (27% [MB] versus 8% [F]; $P < 0.0001$), with added benefit if therapy was initiated prior to the blood culture. The targeted use of MB bottles is warranted for patients at risk for mycobacterial and fungal infections and for infection with *B. pseudomallei*, a common cause of septicemia in Thailand.

Community-acquired bloodstream infections cause substantial morbidity and mortality worldwide, but microbiology capacity and surveillance limitations have challenged good descriptions of pathogen distribution in many regions, including Southeast Asia. As part of a collaboration between the Thailand Ministry of Public Health and the U.S. Centers for Disease Control and Prevention (CDC), we established bloodstream infection surveillance in 2005 using standardized protocols in two rural Thailand provinces. In an effort to maximize yield, blood specimens were inoculated into two culture bottles; one bottle was optimized for aerobic growth (BacT/Alert FA for adults and BacT/Alert PF for children, with peptone-enriched Trypticase soy broth supplemented with brain heart infusion solids and activated charcoal), and the second bottle was optimized for enhanced growth of mycobacteria and other fastidious organisms (BacT/Alert MB, with Middlebrook 7H9 broth with glycerol and sodium polyanethole sulfonate). The detection system and method of signaling for a positive culture are the same for the MB and F bottles, although the MB bottles are incubated longer (up to 42 days) than the F bottles (maximum of 5 days). Routine use of MB culture bottles (or comparable bottles in other blood culture systems) may be indicated in settings with a high prevalence of HIV or tuberculosis. However, these bottles are expensive and, with incubation for up to 42 days required for slow-growing pathogens, can strain the incubator capacity. The routine use of MB bottles for bloodstream infection surveillance and clinical evaluation therefore warrants further evaluation. Data on the yield of different blood culture systems and culture broth formulations in resource-constrained settings are limited (1, 2). We sought to describe the pathogens causing bloodstream infections in rural Thailand and to quantify the added overall yield of routinely inoculating blood for culture into MB bottles and for specific pathogens, including *Burkholderia pseudomallei*, the causative agent of melioidosis, a high-burden disease in Thailand (3).

MATERIALS AND METHODS

Bloodstream infection surveillance. We have conducted enhanced surveillance for bloodstream infections in two rural Thailand provinces since 2005. The Nakhon Phanom province borders Laos in the northeast and is an area in which *B. pseudomallei* is highly endemic (3), while Sa Kaeo

Received 28 July 2014 **Returned for modification** 2 September 2014
Accepted 5 January 2015

Accepted manuscript posted online 14 January 2015

Citation Jorakate P, Higdon M, Kaewpan A, Makprasert S, Yuenprakhon S, Tawisaid K, Dejsirilert S, Whistler T, Baggett HC. 2015. Contribution of the BacT/Alert MB *Mycobacterium* bottle to bloodstream infection surveillance in Thailand: added yield for *Burkholderia pseudomallei*. J Clin Microbiol 53:910–914. doi:10.1128/JCM.02008-14.

Editor: N. A. Ledeboer

Address correspondence to Possawat Jorakate, hqg0@cdc.gov.

* Present address: Melissa Higdon, International Vaccine Access Center, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA.

P.J. and M.H. contributed equally to this work.

doi:10.1128/JCM.02008-14

borders Cambodia in the east and has a lower melioidosis burden. Surveillance is conducted in all 12 hospitals in Nakhon Phanom (1 provincial hospital, 11 community hospitals) and in 8 hospitals in Sa Kaeo (1 provincial hospital, 7 community hospitals) (4, 5).

Blood was collected from the hospitalized patients at clinician discretion, and collection was encouraged for all patients with pneumonia and for children less than 5 years of age with suspected sepsis. Nurses aimed to collect 10 ml of blood for inoculation into the BacT/Alert FA bottle for patients aged >5 years or 4 ml for inoculation into the BacT/Alert PF bottle for children ≤5 years (FA and PF bottles are hereafter referred to as F bottles) and at least 3 ml for inoculation into the BacT/Alert MB bottle. When blood volume was insufficient, inoculation into the F bottle was prioritized. Blood samples collected at community hospitals were transported at 15°C to 30°C for centralized testing at the provincial hospital microbiology laboratory within 24 h.

One milliliter of enrichment fluid (bovine serum albumin, sodium chloride, oleic acid, and saponin in water) was added to each MB bottle upon receipt in the laboratory, and the F and MB bottles were placed into the BacT/Alert 3D automated blood culture system (bioMérieux, USA). The F and MB bottles were incubated at 35°C for up to 5 and 42 days, respectively, or until the instrument signaled a positive result for growth (i.e., alarm positive). Blood from the positive bottles was removed and subcultured onto sheep blood, chocolate, and MacConkey agar plates and incubated for 24 to 48 h at 35°C in 5% CO_2 for the sheep blood and chocolate agar plates and at normal atmosphere for the MacConkey agar plates. Gram staining, biochemical tests, and commercial bacterial identification strips (API strips; bioMérieux, USA) were used to identify positive bacterial cultures by standard microbiology procedures (6). Culture bottles that did not signal a positive result after the specified incubation periods were considered negative without terminal subculture.

The presence of an antimicrobial agent in a patient's serum was assessed by coating filter paper with 20 μl of the patient's serum collected at the time of blood culture and placing the paper on Mueller-Hinton agar inoculated with *Staphylococcus aureus* (strain ATCC 9144) (7). After incubation for 24 h at 35°C, a clear zone around the disc was considered evidence of serum antimicrobial activity.

Data analysis. This analysis included blood cultures performed from May 2005 through April 2012. In order to quantify the contribution of the MB bottles to the detection of blood-borne pathogens, we limited our analyses to patient blood cultures for which both an F and an MB bottle had been placed. We did not have multiple cultures or longitudinal clinical data to confirm whether all organisms were true pathogens, so we relied on previous assessments (8) to categorize organisms as likely pathogens or likely contaminants. A patient's blood culture was considered positive for a likely pathogen if that organism was identified in either of the culture bottles and if the organism was most often a pathogen in prior studies. Common skin and environmental organisms were considered likely contaminants. A list of organisms considered likely contaminants is given in Table 1. For each pathogen, we determined the proportion of total positive results that were confirmed in the MB bottles alone by dividing the number of cultures for which the organism was isolated from only the MB bottle by the total number of positive results. To assess whether the added yield from the MB bottle cultures was attributable to the MB bottle medium (as opposed to simply the added yield of a second bottle), we compared the proportion of cultures that were positive in only the MB bottle to the proportion positive from only the F bottle using McNemar's test with Bonferroni's correction for multiple tests. Starting with an α value of 0.05 and 29 different pathogens for comparison, the new α value after correction was 0.0017 (0.05/29). Thus, statistical significance was defined as a P value of ≤0.0017 (9). We assessed the time to culture positivity, blood volume cultured, and serum antimicrobial activity as potential confounders of the relationship between pathogen yield and bottle type (10).

RESULTS

From May 2005 through April 2012, 63,066 blood cultures were performed with blood inoculated into F and MB culture bottles. Of those, 11,801 (18.7%) signaled a positive result in the BacT/Alert instrument for at least one of the two bottles (Table 1).

Of the 11,801 that signaled positive, a presumed pathogen was isolated from at least 1 of the 2 bottles in 7,296 of the cases (61.8% [and 12.0% of all blood cultures]). A total of 2,859 (24.2%) had no pathogen but had a contaminant in at least one bottle. Among the 63,066 cases, 793 (1.3%) of the F bottle cultures and 1,023 (1.6%) of the MB bottles were alarm positive but had no growth ($P <$ 0.0001). Of the 3,796 pathogens isolated in Sa Kaeo and the 3,833 pathogens isolated in Nakhon Phanom, *Escherichia coli* was most common pathogen in both provinces (29.1% and 27.9%, respectively, of all pathogens) followed by *Klebsiella pneumoniae* (8.7%) and *S. aureus* (6.7%) in Sa Kaeo and *B. pseudomallei* (17.1%) and *K. pneumoniae* (9.2%) in Nakhon Phanom.

Of 7,629 pathogens isolated from 7,296 patient blood cultures, 4,414 (57.9%) were isolated from both the MB and F bottles, 1,589 (20.8%) were isolated from only the F bottle, and 1,626 (21.3%) were isolated from only the MB bottle. Several bacteria, including both Gram-positive (e.g., *Streptococcus pyogenes* and *Streptococcus pneumoniae*) and Gram-negative (e.g., *E. coli*) pathogens, were isolated significantly more often from only the F bottle than from only the MB bottle (Table 1). Mycobacteria were isolated exclusively from the MB bottles, and two fungal species (*Penicillium* species and *Histoplasma capsulatum*) were more commonly isolated from the MB bottles. Among nonmycobacterial pathogens, 17.5% were isolated only from the MB bottles, while 21.8% were isolated only from the F bottles. The percentages of Gram-positive bacteria detected in the MB and F bottles were 74% and 89%, respectively. Compared to the F bottles, the MB bottles contained a lower percentage of Gram-negative bacteria (81% versus 84%, respectively) but a higher percentage of fungi (84% versus 75%, respectively). The mean blood volumes cultured were 7.0 ml (standard deviation [SD], 2.5 ml) in the MB bottles and 9.9 ml (SD, 0.85 ml) in the F bottles for patients aged >5 years. Among those aged ≤5 years, the mean volumes were 3.0 ml (SD, 1.1 ml) in the MB bottles and 4.1 ml (SD, 1.4 ml) in the F bottles. Among nonmycobacterial pathogens, the median times to a positive result were 16 h for the MB bottles and 14 h for the F bottles. There were 169 positive cultures for which the MB bottled yield a nonmycobacterial pathogen after 5 days (corresponding to the maximum incubation period of the F bottle), of which 47 also had the pathogen detected in the F bottle.

The only nonmycobacterial bacterium isolated significantly more commonly in the MB than in the F bottles was *B. pseudomallei*. Of the 837 blood cultures positive for *B. pseudomallei*, 222 (26.5%) were positive only in the MB bottle compared to 69 (8.2%) that were positive only in the F bottle; 65.2% were positive in both bottles. The median (range) times to a positive result for the detection of *B. pseudomallei* were 23 h (5 to 327 h) for the MB bottle and 25 h (2 to 123 h) for the F bottle. Among the *B. pseudomallei* cases detected only in the MB bottle, 78.8% signaled positive within 2 days, and only 2.3% signaled positive after 5 days, when the accompanying F bottle would no longer have been incubating. Among the 837 *B. pseudomallei* cases, 188 (22.5%) were

TABLE 1 Clinically relevant pathogen identifications by blood culture among patients with blood inoculated into BacT/Alert FA and BacT/Alert MB bottles in Sa Kaeo and Nakhon Phanom, Thailand, 2005–2012[a]

Pathogen[b]	Total no. of cultures	Culture result by bottle:						
		MB^+, F^-[c]		MB^-, F^+		MB^+, F^+		
		No.	%	No.	%	No.	%	P[d]
Gram positive								
Enterococcus species	149	33	22.2	55	36.9	61	40.9	0.0190
Staphylococcus aureus	488	50	10.3	82	16.8	356	73.0	0.0053
Streptococcus agalactiae	117	14	12.0	7	6.0	96	82.1	0.1266
Streptococcus bovis	71	3	4.2	26	36.6	42	59.2	<0.0001
Streptococcus pneumoniae	200	16	8.0	67	33.5	117	58.5	<0.0001
Streptococcus pyogenes	173	14	8.1	61	35.3	98	56.7	<0.0001
Streptococcus suis	49	2	4.1	27	55.1	20	40.8	<0.0001
Gram negative								
Acinetobacter species	280	65	23.2	89	31.8	126	45.0	0.0531
Brucella species	8	2	25.0	0	0.0	6	75.0	0.1573
Burkholderia pseudomallei	837	222	26.5	69	8.2	546	65.2	<0.0001
Escherichia coli	2,174	294	13.5	423	19.5	1,457	67.0	<0.0001
Enterobacter species	103	13	12.6	26	25.2	64	62.1	0.0374
Haemophilus influenza	27	1	3.7	26	96.3	0	0.0	<0.0001
Klebsiella pneumoniae	683	84	12.3	123	18.0	476	69.7	0.0067
Moraxella catarrhalis	18	6	33.3	10	55.6	2	11.1	0.3173
Pasteurella species	5	0	0.0	2	40.0	3	60.0	0.1573
Proteus species	93	16	17.2	15	16.1	62	66.7	0.8575
Pseudomonas aeruginosa	169	24	14.2	51	30.2	94	55.6	0.0018
Salmonella enterica subsp. *enterica* serovar Paratyphi A	2	0	0.0	0	0.0	2	100.0	1.0000
Salmonella species	327	39	11.9	80	24.5	208	63.6	0.0002
Salmonella enterica subsp. *enterica* serovar Typhi	8	0	0.0	1	12.5	7	87.5	0.3173
Serratia marcescens	5	0	0.0	3	60.0	2	40.0	0.0833
Acid-fast bacilli								
Mycobacterium tuberculosis	199	199	100.0	0	0.0	0	0.0	
Nontuberculosous mycobacterium	155	155	100.0	0	0.0	0	0.0	
Fungi								
Candida species	97	29	29.9	25	25.8	43	44.3	0.5862
Cryptococcus neoformans	271	28	10.3	42	15.5	201	74.2	0.0943
Histoplasma capsulatum	31	29	93.6	1	3.2	1	3.2	<0.0001
Penicillium species	65	29	44.6	4	6.2	32	49.2	<0.0001
Other pathogens[e]	825	259	31.4	274	33.2	292	35.4	
Total no. of pathogens	7,629	1,626	21.3	1,589	20.8	4,414	57.9	<0.0001
Total no. of nonmycobacterial pathogens	7,275	1,272	17.5	1,589	21.8	4,414	60.7	<0.0001

[a] BacT/Alert FA (PF for children <5 years old) and BacT/Alert MB (F and MB) bottles were used. The total no. of blood cultures was 63,066.
[b] The following organisms were considered likely contaminants and were not included in the analysis: *Streptococcus viridans* group, *Corynebacterium urealyticum*, other *Corynebacterium* species, other *Bacillus* species, coagulase-negative *Staphylococcus*, other Staphylococcus species, and *Aerococcus* species.
[c] +, result for the indicated bottle was positive; −, result for the indicated bottle was negative.
[d] McNemar's test comparing the proportions of each pathogen detected in the MB bottle only to the proportion detected in the F bottle only. With Bonferroni's multiple test correction, statistical significance was considered at a P value of <0.0017.
[e] Other pathogens included *Aeromonas hydrophila*, *Aeromonas* species, *Alcaligenes faecalis*, other *Alcaligenes* species, *Aspergillus* species, *Bacillus cereus*, *Bordetella bronchiseptica*, *Brevundimonas* species, *Burkholderia cepacia*, other *Burkholderia* species, *Citrobacter* species, *Corynebacterium jeikeium*, other *Cryptococcus* species, other *Escherichia* species, other *Haemophilus* species, other *Klebsiella* species, *Listeria monocytogenes*, other *Moraxella* species, other *Neisseria* species, *Providencia* species, other *Pseudomonas* species, *Ralstonia pickettii*, *Serratia* species, *Shigella sonnei*, *Stenotrophomonas maltophilia*, other *Streptococcus* species, *Vibrio cholera*, other *Vibrio* species, unspecified yeast, and mold.

in patients with evidence of prior antibiotic use, determined by the presence of serum antimicrobial activity (Table 2).

Among all 63,066 patients, 46,666 patients had antibiotic activity information available, and *B. pseudomallei* was isolated from 768 (1.2%) MB bottles and 615 (1.0%) F bottles ($P <$ 0.0001). The difference in *B. pseudomallei* isolation by bottle type was limited to patients with evidence of prior antibiotic use. Among the 12,091 patients with prior antibiotic use, the MB bottles were nearly twice as likely as the F bottles to yield *B. pseudomallei* (1.47% versus 0.81%, respectively; $P < 0.0001$; Table 2). Among the 34,575 patients without prior antibiotic use, the *B. pseudomallei* isolation rates were similar between the

TABLE 2 Pathogen isolation[a] in BacT/Alert FA and BacT/Alert MB bottles[b] according to the presence of antimicrobial activity in sera from patients in Sa Kaeo and Nakhon Phanom, Thailand, 2005–2012

	Pathogen yield result for:											
	ABX[c,d] present (n = 12,091)						ABX[c,d] not present (n = 34,575)					
	F bottle		MB bottle		Difference in yield (MB − F)		F bottle		MB bottle		Difference in yield (MB − F)	
Pathogen	No.	%	No.	%	%	McNemar's P	No.	%	No.	%	%	McNemar's P
Burkholderia pseudomallei[e]	98	0.81	178	1.47	0.66	<0.0001	295	0.85	311	0.90	0.05	0.1131
Escherichia coli	168	1.39	163	1.35	−0.04	0.5737	1,128	3.26	1,050	3.04	−0.22	0.0002
Klebsiella pneumoniae	59	0.49	52	0.43	−0.06	0.0896	363	1.05	332	0.96	−0.09	0.0068
Salmonella species[f]	25	0.21	19	0.16	−0.05	0.0143	179	0.52	158	0.46	−0.06	0.0127
Staphylococcus aureus	31	0.26	20	0.17	−0.09	0.0076	261	0.75	245	0.71	−0.04	0.0455
Streptococcus pneumoniae	4	0.03	4	0.03	0.00	1.0000	110	0.32	75	0.22	−0.10	<0.0001
Streptococcus pyogenes	5	0.04	1	0.01	−0.03	0.1025	102	0.30	78	0.23	−0.07	0.0001
Total pathogens	390	3.2	437	3.6	0.04	0.0022	2,438	7.1	2,249	6.5	0.6	<0.0001

[a] Selected subset of commonly identified pathogens.
[b] BacT/Alert FA (PF for children <5 years old) and BacT/Alert MB (F and MB) bottles were used.
[c] ABX, antimicrobial activity.
[d] ABX presence was determined by antimicrobial activity in patient sera at the time of blood collection (4).
[e] Numbers in this table are lower than those in Table 1, because antibiotic activity data were not available for all cases.
[f] All nontyphoidal *Salmonella* species.

MB and the F bottles (0.90% versus 0.85%, respectively; P = 0.1131). Serum antimicrobial activity decreased the overall yield of several pathogens that were more commonly isolated from the F bottles and obscured the relationship between the yield and the bottle type (selected pathogens are shown in Table 2).

DISCUSSION

Based on 7 years of bloodstream infection surveillance in two rural Thailand provinces with routine inoculation of blood culture bottles to enhance the detection of mycobacteria and other fastidious pathogens (MB bottles) in addition to standard aerobic bottles (F bottles), the most common pathogens were *E. coli*, *B. pseudomallei*, *K. pneumoniae*, and *S. aureus*. Because only 79% of the overall pathogen yield was accounted for by a single F bottle (83% of nonmycobacterial pathogens), we can infer that using two bottles improved the yield overall. However, any added yield specifically due to the MB bottles was limited to a few pathogens. In addition to the detection of mycobacteria and some fungi, the MB bottles improved the detection of *B. pseudomallei* detection.

Our data support the conclusion that inoculating two blood culture bottles improves the pathogen yield over that of just one bottle, which has been shown previously (1, 11). However, for the majority of pathogens, the percentage of isolates detected by the MB bottle was similar to or lower than the percentage of isolates detected by the F bottle, suggesting that the added yield was not attributable to properties inherent to the MB bottle and that inoculating a second F bottle would have increased the overall yield at least as much as an MB bottle. F bottles have the added benefit of being substantially less expensive than MB bottles. Therefore, for most pathogens, replacement of the MB bottle with a second F bottle would cut costs and lessen the burden on the laboratory without hindering (and possibly improving) pathogen detection. Our findings confirm that MB bottles are necessary for isolating mycobacteria and likely improve the detection of certain fungi, such as *H. capsulatum* and *Penicillium* species.

Our findings also support the observation that the isolation of *B. pseudomallei*, a common cause of septicemia in Thailand, is enhanced by the use of MB bottles. This unexpected effect was limited to patients with evidence of antibiotic receipt before sample collection; no significant associations between bottle type and *B. pseudomallei* yield were found among patients without prior antibiotic use. We hypothesize that the added *B. pseudomallei* yield of the MB bottles is attributable to the presence of saponin in the enrichment medium added to the MB bottle just prior to inoculation and incubation. Saponin is a lytic agent used for the disruption of red blood cells to release intracellular bacteria like *B. pseudomallei* into the culture broth (12, 13). Other studies have shown that saponin has no effect on bacterial growth (14). However, it is not clear why the impact of saponin would be limited to patients pretreated with antibiotics. The MB and F bottles both contained sodium polyanethol sulfonate to inhibit aminoglycoside and polymyxin antibiotics (15).

These results may have important clinical implications in settings where *B. pseudomallei* is endemic. In Thailand, patients with suspected sepsis are typically treated with broad-spectrum antibiotics but not always with antibiotics that would cover *B. pseudomallei* (i.e., ceftazidime or imipenem). Our findings suggest that culturing blood in an MB bottle (in addition to an F bottle) may improve *B. pseudomallei* isolation in the presence of antibiotic pretreatment and thereby inform a change to an appropriate antibiotic regimen for patients with melioidosis. Our findings should be interpreted in the light of some limitations. Although we found that the pathogen yield was improved with two culture bottles, we cannot exclude the possibility that this increased yield was due to the overall increase in the volume of blood cultured (16) as opposed to inoculation into a second culture bottle. The retrospective nature of this analysis limited our ability to control for unmeasured factors that might have confounded the relationship between the bottle type and the pathogen yield, such as patient clinical characteristics. For example, more severely ill patients may have been more likely to be pretreated with antibiotics

and to have higher bacterial loads, possibly explaining the increased *B. pseudomallei* yield among patients with prior antibiotic receipt. The lack of prospectively collected clinical information also may have limited our ability to distinguish true pathogens from likely contaminants and led to misclassification. Some organisms categorized as likely contaminants, such as coagulase-negative staphylococci and viridans group streptococci, can be true pathogens in certain clinical situations (17, 18). Our analysis was limited to patients who had blood inoculated into both F and MB bottles, and these patients may have differed from patients who only had one culture bottle inoculated, limiting the generalizability of our findings on the overall pathogen distribution. The generalizability of some of our findings may also be limited to Thailand, where pathogen distribution, especially of *B. pseudomallei*, may differ from that in other settings.

Our findings demonstrate the value of two culture bottles and that, for most pathogens, the F bottle yield is similar to or exceeds that of the MB bottles. The MB bottle culture was necessary for detecting mycobacteria (19) and obtaining an improved yield for *B. pseudomallei*. Our results support the targeted use of MB bottles for immunocompromised patients who may be at increased risk for mycobacteremia and invasive fungal infections and when *B. pseudomallei* infection is suspected. Our results and those of Archibald et al. (20) support the clinical utility of including MB bottles, not only for the diagnosis of melioidosis in patients already treated with antibiotics but also in populations with a high prevalence of undiagnosed HIV.

ACKNOWLEDGMENTS

Support for this project was provided by the CDC Foundation, the Pneumococcal Vaccines Accelerated Development and Introduction Plan (PneumoADIP) (funded by the GAVI Alliance based at the Johns Hopkins Bloomberg School of Public Health), and the Global Disease Detection program of the U.S. Centers for Disease Control and Prevention.

For their contributions to this work, we thank Leonard Peruski, Sathapana Naorat, Prabda Prapasiri, Prasong Srisaengchai, and Apiwat Lapamnouysup (Global Disease Detection Regional Center, Thailand Ministry of Public Health-U.S. Centers for Disease Control and Prevention [CDC] Collaboration), Peera Areerat and Pichai Thongtaradol (Nakhon Phanom Provincial Health Office), and Somyote Srijaranai and Chaiyapruck Chalernjit (Sa Kaeo Provincial Health Office).

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

REFERENCES

1. **Archibald LK, Dobbie H, Kazembe P, Nwanyanwu O, McKnight C, Byrne T, Addison RM, Bell M, Reller LB, Jarwis WR.** 2001. Utility of paired Bactec NYCI/F LYTIC blood culture vials for detection of bacteremia, mycobacteremia, and fungemia. J Clin Microbiol **39:**1960–1962. http://dx.doi.org/10.1128/JCM.39.5.1960-1962.2001.
2. **Archibald LK, McDonald LC, Addison RM, McKnight C, Byrne T, Dobbie H, Nwanyanwu O, Kazembe P, Reller LB, Jarwis WR.** 2000. Comparison of Bactec MYCO/F LYTIC and Wampole Isolator 10 (lysis-centrifugation) systems for detection of bacteremia, and fungemia in a developing country. J Clin Microbiol **38:**2994–2997.
3. **Bhengsri S, Lertiendumrong J, Baggett HC, Thamthitiwat S, Chierakul W, Tisayaticom K, Tanwisaid K, Chantra S, Kaewkungwal J.** 2013. Economic burden of bacteremic melioidosis in eastern and northeastern, Thailand. Am J Trop Med Hyg **89:**369–373. http://dx.doi.org/10.4269/ajtmh.13-0148.
4. **Baggett HC, Peruski LF, Olsen SJ, Thamthitiwat S, Rhodes J, Dejsirilert S, Wongjindanon W, Dowell SF, Fischer JE, Areerat P, Sornkij D, Jorakate P, Kaewpan A, Prapasiri P, Naorat S, Sangsuk L, Eampokalap B, Moore MR, Carvalho G, Beall B, Ungchusak K, Maloney SA.** 2009. Incidence of pneumococcal bacteria requiring hospitalization in rural Thailand. Clin Infect Dis **48**(suppl 2)**:**S65–S74. http://dx.doi.org/10.1086/596484.
5. **Bhengsri S, Baggett HC, Jorakate P, Kaewpan A, Prapasiri P, Naorat S, Thamthitiwat S, Tanwisaid K, Chantra S, Salika P, Dejsirilert S, Peruski LF, Maloney SA.** 2011. Incidence of bacteremic melioidosis in eastern and northeastern Thailand. Am J Trop Med Hyg **85:**117–120. http://dx.doi.org/10.4269/ajtmh.2011.11-0070.
6. **Patrick RM, Ellen JB, James HJ, Michael AP, Robert HY.** 2003. Manual of clinical microbiology, 8th ed, vol 1. ASM Press, Washington, DC.
7. **Driscoll AJ, Bhat N, Karron RA, O'Brien LK, Murdoch DR.** 2012. Disk diffusion bioassays for the detection of antibiotic activity in body fluids: applications for the pneumonia etiology research for child health project. Clin Infect Dis **54**(suppl 2)**:**S159–S164. http://dx.doi.org/10.1093/cid/cir1061.
8. **Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, Reller LB.** 1997. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology and outcome of bacteremia and fungemia in adults. Clin Infect Dis **24:**584–602. http://dx.doi.org/10.1093/clind/24.4.584.
9. **Donna FS, Steven MT.** 1998. Statistics in public health: quantitative approaches to public health problems. Oxford University Press, New York, NY.
10. **Rhodes J, Hyder JA, Peruski LF, Fisher C, Jorakate P, Kaewpan A, Dejsirilert S, Thamthitiwat S, Olsen SJ, Dowell SF, Chantra S, Tanwisaid K, Maloney SA, Baggett HC.** 2010. Antibiotic use in Thailand: quantifying impact on blood culture yield and estimates of pneumococcal bacteremia incidence. Am J Trop Med Hyg **83:**301–306. http://dx.doi.org/10.4269/ajtmh.2010.09-0584.
11. **Riley JA, Heiter BJ, Bourbeau PP.** 2003. Comparison of recovery of blood culture isolates from two BacT/ALERT FAN aerobic blood culture bottles with recovery from one FAN aerobic bottle and one FAN anerobic bottle. J Clin Microbiol **4:**213–217. http://dx.doi.org/10.1128/jcm.41.1.213-217.2003.
12. **Allwood EM, Devenish RJ, Prescott M, Adler B, Boyce JD.** 2011. Strategies for intracellular survival of *Burkholderia pseudomallei*. Front Microbiol **2:**170. http://dx.doi.org/10.3389/fmicb.2011.00170.
13. **Jones AL, Beveridge TJ, Woods DE.** 1996. Intracellular survival of *Burkholderia pseudomallei*. Infect Immun **64:**782–790.
14. **Sergey Z, Harisha R, Jonas H, Sahar A, Hjamar B, Aman R.** 2011. Bacteria isolation from whole blood for sepsis diagnostics. Presented at the 15th Intl Conf Miniaturized Syst Chem Life Sci, Seattle, WA, 2–6 October 2011.
15. **Edberg SC, Bottenbley CJ, Gam K.** 1976. Use of sodium polyanethol sulfonate to selectively inhibit aminoglycoside and polymyxin antibiotics in a rapid blood level antibiotic assay. Antimicrob Agents Chemother **9:**414–417.
16. **Bouza E, Sousa D, Rodriguez-Creixems M, Lechuz JG, Munoz P.** 2007. Is the volume of blood cultured still a significant factor in the diagnosis of bloodstream infections? J Clin Microbiol **45:**2765–2769. http://dx.doi.org/10.1128/JCM.00140-07.
17. **Hall KK, Lyman JA.** 2006. Updated review of blood culture contamination. Clin Microbiol Rev **19:**788–802. http://dx.doi.org/10.1128/CMR.00062-05.
18. **Kirn TJ, Mirrett S, Reller LB, Weinstein MP.** 2014. Controlled clinical comparison of BacT/Alert FA Plus and FN Plus blood culture media with BacT/Alert FA and FN blood culture media. J Clin Microbiol **52:**839–843. http://dx.doi.org/10.1128/JCM.03063-13.
19. **Crump JA, Tanner DC, Mirrett S, McKnight CM, Reller LB.** 2003. Controlled comparison of Bactec 13A, MYCO/F LYTIC, BacT/ALERT MB, and Isolator 10 systems for detection of mycobacteremia. J Clin Microbiol **41:**1987–1990. http://dx.doi.org/10.1128/JCM.41.5.1987-1990.2003.
20. **Archibald LK, McDonald LC, Rheanpumikankit S, Tansuphaswadikul S, Chaovanich A, Eampokalap B, Banerjee SN, Reller LB, Jarvis WR.** 1999. Fever and human immunodeficiency virus infection as sentinels for emerging mycobacterial and fungal bloodstream infections in hospitalized patients >15 years old, Bangkok. J Infect Dis **180:**87–92. http://dx.doi.org/10.1086/314836.