A comparison of lysis centrifugation, pour plate, and conventional blood culture methods in the diagnosis of septicaemic melioidosis

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Abstract

Aims—To determine whether quantitative blood culture methods could improve the diagnosis of septicaemic melioidosis.
Methods—A comparison of conventional broth based blood cultures, a pour plate method, and a commercial lysis centrifugation (Isolator 10™) blood culture system was conducted in 71 Thai patients with severe melioidosis. The time to identification of *B pseudomallei* was recorded for each method.
Results—42 patients (59%) were septicaemic. Compared with conventional blood culture, the Isolator and pour plate methods had sensitivities of 81% and 61%, respectively. The median times to a positive culture were: Isolator 39.3 hours, pour plates 45.5 hours, broth culture 61.8 hours (p < 0.001 Isolator *v* broth). There was a significant inverse correlation between Isolator tube or pour plate quantitative counts and time to detection (*r* = −0.44 and −0.57, respectively). Mortality was higher in patients who were septicaemic.
Conclusions—Routine use of one of these quantitative methods, in addition to conventional broth culture, may lead to earlier diagnosis of septicaemic melioidosis.

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Melioidosis, an infection caused by the environmental saprophyte *Burkholderia pseudomallei*, is endemic to parts of Southeast Asia and northern Australia.[1] Melioidosis is a major cause of human morbidity and mortality in northeast Thailand. In Ubon Ratchathani, close to the borders of both Laos and Cambodia, melioidosis is the cause of 20% of cases of community acquired septicaemia, and 40% of all deaths in this group of patients.[2] The annual disease incidence in this province has been estimated at 4.4 cases per 100 000.[3] Antibiotic treatment is available, although expensive, but in-hospital mortality in severe disease remains approximately 40%. Many patients with melioidosis die within 48 hours of reaching hospital,[4] often before identification of the causative organism can be achieved. Commonly used empirical antibiotic regimens for presumed septicaemia, such as penicillin–aminoglycoside combinations, have little activity against *B pseudomallei*. Appropriate treatment depends therefore on a high index of clinical suspicion or rapid identification of the causative organism. Although a rapid presumptive diagnosis can be made using a specific immunofluorescence (IF) test,[5] this relies on the availability of suitable specimens (such as sputum, urine, or pus) and an immunofluorescence microscope. Approximately 60% of patients with melioidosis are septicaemic on admission to hospital.[3] Blood cultures may become positive within 24 hours, but subculture and identification of the organism requires further time. More rapid methods for diagnosis and confirmation of septicaemic melioidosis would facilitate earlier appropriate antibiotic treatment.

We report a study comparing speed of isolation of *B pseudomallei* from conventional broth based blood cultures, a pour plate method, and the Wampole Isolator 10™ lysis centrifugation blood culture system, in adult Thai patients with severe melioidosis.

Methods

Adult patients (aged ≥ 15 years) admitted to Sappasitprasong Hospital, Ubon Ratchathani, with suspected severe melioidosis were studied if they were eligible for entry into a separate open, randomised trial of ceftazidime versus imipenem treatment.[6] Blood cultures (two or three 5 ml blood samples, each inoculated into broth media) were collected at entry, together with three × 1 ml sterile heparinised blood samples[7] collected for quantification of bacteraemia. Cultures of sputum, throat swabs, urine, and pus (from wounds, other skin lesions, or aspirated from visceral abscesses) were performed. Blood culture bottles (TSC, Heywood, Lancashire, UK) contained 45 ml brainheart infusion (BHI) broth with sodium polyanethol sulphonate (SPS). For this study, an additional 10 ml of blood was inoculated aseptically into a pre-evacuated Isolator 10 lysis centrifugation blood culture tube (Unipath, Basingstoke, Hampshire, UK). The Isolator 10 tubes contain SPS, saponin, and polypropylene glycol in aqueous solution.

Broth blood culture bottles were vented (using BCB Vent/Sub units; Difco, West Molesey, Surrey, UK) and incubated unshaken in air for seven days at 37°C. The bottles were inspected daily and Gram stains performed on smears prepared at 12–24 hours and 36–48 hours, or when visible growth occurred. Bottles were subcultured routinely onto horse blood agar at 12–24 and 36–48 hours and after seven days of incubation, and were subcultured on days 3–6 if there was any suggestion of visible growth (that is, cloudy broths).

Table 1 Comparison of times to first positive culture with different blood culture methods

Method	Number positive	Median time to positive (h)	Range (h)	Mean (SD) time to positive (h)
BHI broth	42	61.8	35 to 205.5	78.9 (51.2)
Isolator™	34	39.3	16 to 64.5	37.2 (11.7)
Pour plate	25	45.5	16 to 91.5	45.5 (12.8)

BHI, brain–heart infusion.

Pour plates were prepared by mixing 1 ml of heparinised blood with 19 ml of molten (50°C) sterile Oxoid Columbia agar (Unipath) in a sterile Petri dish, as previously described.[7]

Isolator tubes were inverted at least 10 times after inoculation to ensure adequate mixing. They were transported immediately to the microbiology laboratory and after a minimum of 10 minutes post-inoculation, the tubes were centrifuged at 1500 g and 4°C for 30 minutes (unbraked). The supernatant (approximately 9 ml) was removed aseptically to leave 1 ml fluid plus sediment. The sediment was homogenised using a sterile pipette, and 0.5 ml volumes were inoculated onto both horse blood and Ashdown's selective agar plates.[8]

All agar plates were incubated for four days at 37°C in air and examined daily at a set time. Colony counts were performed on relevant plates to allow calculation of the number of colony forming units per millilitre (cfu/ml) of blood.

Statistical analyses were performed using the statistical computing package SPSS for Windows 7.5 (SPSS Inc, Chicago, Illinois, USA).

Results

Between August 1996 and November 1997, 82 patients were enrolled into the study. Of these, 71 (86.6%) were proved subsequently to have melioidosis (that is, cultures were positive for *B pseudomallei*) and 42 patients (59.2%) had positive blood cultures. Three broth culture bottles were inoculated in 35 of the 42 septicaemic patients. Of these, 21 cultures (60%) were positive in all three bottles, while eight (23%) and six (17%) were positive in only one or two bottles, respectively. Two bottles were collected in six patients; five were positive in both bottles. In the final patient only one bottle was collected. Thus all bottles were positive in 27 patients (63%). Gram negative rods were seen in stained routine smears from blood culture broths in only four patients (9.5%).

B pseudomallei was isolated from the Isolator tube (limit of detection 0.1 cfu/ml) in 34 (47.9%) of the 71 patients, while 25 (35.3%) of

Table 2 Quantitative bacterial counts in peripheral blood by Isolator™ or pour plate methods and related mortality (all blood culture positive patients, n=42)

	Count (cfu/ml)					
	0	<1	1–10	11–40	41–100*	>100
Pour plates						
n (%)	16 (39.0)	0	10 (24.4)	3 (7.3)	7 (17.1)	5 (12.2)
Mortality (%)	7 (43.8)	–	5 (50.0)	3 (100)	6 (85.7)	5 (100)
Isolator™						
n (%)	8 (19.0)	6 (14.3)	16 (38.1)	5 (11.9)	7 (16.7)	
Mortality (%)	3 (37.5)	4 (66.7)	8 (50.0)	4 (80.0)	7 (100)	

cfu, colony forming unit.
*Count > 40 cfu/ml for Isolator.

69 patients had positive pour plates (limit of detection 0.3 cfu/ml). Two patients did not have pour plate cultures performed. All patients with either positive pour plates or Isolators were also blood culture positive by the standard broth method. Thus 81.0% of bacteraemic patients (34 of 42) were also positive using the Isolator tube and 61.0% (25 of 42) had positive pour plates. All the septicaemic patients (n = 6) in whom only two broth culture bottles were collected (that is, 10 ml of blood were cultured) were also Isolator positive (100%). Eight patients were Isolator tube positive when pour plate cultures were negative (that is, they had counts of 0.1–0.3 cfu/ml); thus the sensitivity of pour plates compared with the Isolator was 75.8%. The pour plates from one patient with *B pseudomallei* cultured from the Isolator were contaminated and unreadable.

TIME TO FIRST POSITIVE CULTURE

Results for time to recognition of a positive culture for each method are shown in table 1. This was the time at which a microbiological diagnosis of melioidosis could be made by identification of *B pseudomallei*. In the vast majority of cases this was when colonies were available on agar media for further tests such as a specific latex agglutination test. The median time to first recognition of a positive culture for the Isolator was 39.3 hours, compared with 61.8 hours for the broth culture method (p < 0.001, Wilcoxon signed rank test) and 45.5 hours for pour plates (p = 0.003). The pour plate method was also significantly faster than BHI blood culture broths (45.5 hours v 61.8 hours; p = 0.02).

Colonies were available on solid media within 24 hours using the Isolator in nine cases (21.4% of all positive blood cultures), from pour plates in one case, and from blood culture broths in none. At 48 hours the numbers were 32 (76.2%), 22 (52.4%), and 18 (42.9%) cases, respectively.

Of the four patients in whose blood culture broths bacteria were seen on Gram stain, two were detected within 24 hours and a third within 48 hours. In only one case were Gram negative rods seen in broth cultures before the Isolator cultures became positive (17 v 41 hours).

COUNTS

Plate count details for the Isolator and the pour plate methods are shown in table 2. Seven of the 34 positive patients by the Isolator method had very high counts, with at least 40 cfu/ml of blood (that is, > 200 cfu/plate), but growth was too confluent to allow exact counts. The same applied to two pour plate positive patients (> 200 cfu/ml). The Isolator tube method was capable of detecting bacteraemia at lower counts than the pour plate method. However, Isolator tube counts (per ml of blood) were significantly lower than pour plate counts from the same patients, where these could be compared (p = 0.015, Wilcoxon signed rank test; n = 20). There was a good positive correlation between the counts in positive cases for the two methods (Spearman rank correlation coefficient (r) = 0.89, p < 0.001), but at the
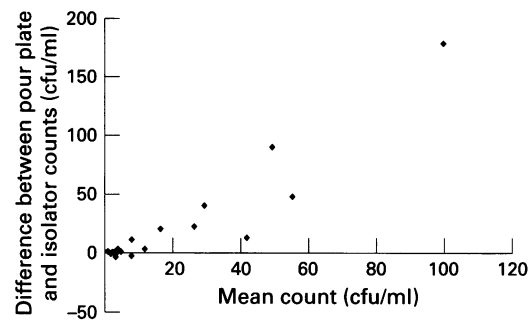
Figure 1 Discrepancies between pour plate and Isolator bacterial counts. Each point represents one patient. The figure shows the relation between the mean quantitative count for both methods combined and the difference in counts between each method, in individual patients. Pour plates generally produced higher counts; at high mean counts the differences were more pronounced.

higher counts the differences in counts were more marked. The discrepancies between the two methods are shown in fig 1. For higher mean counts, these differences between the two methods are more apparent. Overall, the pour plate method gave counts that were a median of 2.5 cfu/ml higher than the Isolator (range −4 to 179 cfu/ml, interquartile range −0.3 to 26.5).

There was also a positive correlation between the number of positive broth culture bottles and bacterial counts (Isolator: $r = 0.590$, p < 0.001; pour plate: $r = 0.422$, p = 0.006).

CORRELATION BETWEEN QUANTITATIVE COUNTS AND TIME TO DETECTION

There was a significant inverse correlation between Isolator tube quantitative counts and time to detection ($r = -0.44$, p = 0.01), that is, higher counts were associated with more rapid detection times. This was also true of pour plates ($r = -0.57$, p = 0.003). There were also significant inverse correlations between Isolator tube or pour plate quantitative counts and times to detection of positive blood culture broth results ($r = -0.72$, p < 0.001; and $r = -0.59$, p < 0.001, respectively).

MORTALITY

Overall 31 of the 71 patients (43.7%) with melioidosis died. Mortality for patients with positive blood cultures was 61.9% (26 of 42) and for patients who were blood culture negative it was 17.2% (five of 29) (p < 0.001). Mortality for patients with *B pseudomallei* isolated from the Isolator tube was 67.6% overall and for pour plate positive patients it was 76.0%. For the Isolator there was no significant difference in mortality between positive and negative patients (p = 0.22, Fisher's exact test), although mortality was significantly higher in those patients with Isolator counts of at least 10 cfu/ml (p = 0.015). Mortality appeared higher in those patients whose pour plates were positive than in those with negative plates, but this did not achieve statistical significance (p = 0.079, χ^2 test). Mortality related to peripheral blood quantitative bacterial counts for each method is shown in table 2.

Discussion

The Isolator 10 lysis centrifugation tube has been shown in previous studies to have comparable sensitivity to conventional blood cultures for recovery of a wide range of organisms, although there have been some reports of reduced recovery of pseudomonads.[9][10] However, in terms of speed of diagnosis, lysis centrifugation has been reported to allow more rapid identification of organisms.[11]

There have been few studies of different blood culture systems in melioidosis, where speed in identifying the presence of *B pseudomallei* is vital. Delay in diagnosis and thus appropriate treatment is a major factor contributing to mortality. *B pseudomallei* grows well in most aerobic media, albeit more slowly than many bacterial pathogens. Quantitative bacterial counts in peripheral blood, using a pour plate method, have been correlated with outcome in melioidosis[7] and thus provide prognostic information, although we did not report on speed of detection in this earlier study. Experience with a lysis centrifugation blood culture method in melioidosis has not been reported previously. The BacT/Alert automated blood culture system has been shown to detect positive blood culture bottles within 24 hours in 62.5% of septicaemic melioidosis cases,[12] but additional incubation time (24–48 hours) was needed before colonies were available for identification. It is possible that routine use of an automated broth blood culture system in the present study may have improved detection times, but such a facility was not available, nor are automated systems often available, in those areas of rural southeast Asia where melioidosis is endemic. In a different series, standard BHI broth manual subcultures at 12–24 hours and 36–48 hours were positive in 52.3% and 80.8% of cases, respectively,[13] but again further incubation time was necessary to confirm isolation of *B pseudomallei*. Routine Gram staining of smears prepared from the blood culture broths, which might allow earlier recognition of Gram negative rods, was not reported in either of these two studies. Thus in practice it was a minimum of 36 hours before colonies were available on solid media and a microbiological diagnosis could be made. Further diagnostic delays are introduced if broth subcultures are not performed at 12–24 hours or if daily inspection and subculture of cloudy broths are not routine procedures. If a Gram stain and specific immunofluorescence test were available, as in our laboratory, a diagnosis of melioidosis could be made confidently at an earlier stage. However, direct immunofluorescence is not yet widely available and would be very costly and time consuming if routinely applied to all blood cultures growing Gram negative rods. Thus more rapid blood culture methods are still required.

The Isolator tube and pour plate methods used in this study had overall sensitivities in septicaemic melioidosis of 81% and 61%, respectively, compared with conventional brain–heart infusion broth cultures. This reduced sensitivity means they could not replace

conventional broth culture, but it must be balanced against the potential for improved speed of isolation and identification of *B pseudomallei*. Both quantitative methods provided significantly earlier detection times (median times 39.3 and 45.5 hours, respectively) in septicaemic melioidosis. All the Isolator positive cases had been detected by 64.5 hours, very close to the median time to detection for conventional broths of 61.8 hours. Detection times were faster with higher peripheral blood bacterial counts for all three methods.

Different volumes of blood (10 *v* 3 ml) were cultured in the two quantitative methods, and this probably explains the difference in sensitivity between them. As might be expected, the Isolator was more sensitive in detecting the presence of bacteraemia than pour plates. It was less sensitive overall than standard broth cultures, where 10 or 15 ml blood were usually cultured, but as the Isolator method lacks an enrichment phase this is not surprising.

Bacterial counts for both quantitative methods correlated well with both the time to detection of positive broth cultures and the number of positive bottles. Although there was good correlation between the two quantitative methods, the pour plate method consistently gave higher quantitative counts. The reason for this is not clear, but clumping of organisms may occur during centrifugation.

Pour plates are an agar incorporation method of quantitative bacterial culture. By its very nature this method is likely to slow down the process of recovery of *B pseudomallei*, as the organism is an obligate aerobe and the characteristic colonial features seen with surface growth are lost. However, although slower than the Isolator and considerably less sensitive than either of the other two methods, the pour plates in our study provided a significantly more rapid method of isolation of *B pseudomallei* colonies than did conventional BHI broths. This pour plate method is very simple and is less technically demanding than the Isolator. It is also much cheaper than the Isolator method (costs per blood tube being approximately US $0.10 and $10.00 respectively, compared with $1.60 per conventional blood culture bottle). The considerable expense involved in routine use of Isolator tubes may put them beyond the budget of many hospitals in the rural tropics, and pour plates are an attractive alternative. Furthermore, the difference between median times to detection for these two methods, although significant, was only six hours, compared with another 16–22 hours for conventional broths (the equivalent of another working day).

We are unable to show from this study that the use of either quantitative method resulted in improved patient outcome, as all patients, by definition, were suspected of having acute severe melioidosis and were immediately started on treatment appropriate for this disease (ceftazidime or imipenem). Thus earlier microbiological confirmation of the diagnosis did not influence antibiotic treatment in the research setting. However, in an endemic area, where empirical regimens for community acquired septicaemia may not be effective

against *B pseudomallei*, more rapid microbiological diagnosis should result in an earlier switch to a more appropriate regimen.

This study shows that more rapid detection of septicaemic melioidosis is possible with the use of either a pour plate or lysis centrifugation blood culture system. The pour plate method is rapid and very cheap, but missed almost 40% of septicaemic patients. The Isolator lysis centrifugation method is also rapid, but is expensive and missed approximately 20% of patients, compared with conventional brainheart infusion broth culture of 15 ml of blood. These quantitative methods also provide important prognostic information—they are most likely to be positive in those patients with the worst prognosis. The conventional broths are slow in comparison but must remain the gold standard method. Quantitative methods should thus augment rather than replace conventional broth culture in patients with suspected septicaemic melioidosis. We recommend the use of either of these quantitative methods alongside conventional broth blood culture. The Isolator tube is the preferred option owing to its greater sensitivity, but costs may dictate the use of the pour plate method in many areas where melioidosis is endemic. This should result in earlier appropriate treatment being offered to patients.

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