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IDENTIFICATION OF BACTERIA

Monoclonal antibody-based rapid identification of *Burkholderia pseudomallei* in blood culture fluid from patients with community-acquired septicaemia

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A monoclonal antibody-based latex agglutination (MAb-LA) test was employed for the rapid identification of *Burkholderia pseudomallei* in blood culture fluid from patients with community-acquired septicaemia. These patients were admitted to 12 hospitals in the northeastern part of Thailand which is a region known to be endemic for melioidosis. Blood samples were collected and immediately added to the blood culture bottles which were incubated in either automated (five hospitals) or manual (seven hospitals) culture systems. Of a total of 1369 culture-positive specimens, 204 specimens were culture-positive for *B. pseudomallei.* Of those, 194 (95%) were positive by MAb-LA and the type of blood culture system did not affect positivity rates. The performance of the MAb-LA test on these specimens was highly satisfactory compared with culture detection and confirmation by biochemical test, with 95.1% sensitivity, 99.7% specificity and 98.8% and 99.2% for positive and negative predictive values, respectively. The method described is highly reproducible, simple to perform even by inexperienced laboratory personnel and does not require expensive or elaborate equipment.

Introduction

Burkholderia pseudomallei is the causative agent of melioidosis, which is an important community-acquired septicaemia in the northeastern part of Thailand [1]. The management of patients with this potentially fatal septicaemic infection depends largely on rapid diagnosis. It has been reported that *c*. 50% of the patients with acute septicaemic melioidosis die within 48 h after admission [2]. Therefore, the earlier the diagnosis is made, the higher the chance of survival. The current diagnostic method for melioidosis is bacterial culture [3, 4]. A molecular approach based upon DNA hybridisation and PCR has been reported with satisfactory results in laboratory tests by several groups of investigators, but in a recent communication the results from a clinical setting were rather disappointing when compared with the classical culture method currently used as the 'gold standard' [5]. Several immunological tests have been developed, but all still need improvement [6]. An earlier report described a highly satisfactory test based on the use of a monoclonal antibody latex agglutination (MAb-LA) test for the identification of *B. pseudomallei* in blood culture fluid taken from automated culture systems in two major hospitals in the endemic northeastern part of Thailand [7]. In collaboration with the Ministry of Public Health of Thailand, the present study extended this evaluation to cover 12 hospitals located over the entire melioidosis endemic area, where both automated and manual culture systems were used.

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Materials and methods

Collection and processing of clinical specimens

A total of 1369 culture-positive blood specimens was collected from patients clinically suspected of having community-acquired septicaemia, by standard aseptic techniques. These patients were admitted to 12 hospitals located throughout the melioidosis endemic area in the northeastern part of Thailand. The study spanned a 5-month period during the rainy season (July

1998–Nov. 1998), when melioidosis usually occurs [8]. The blood was cultured immediately, by either a manual or automated (BacT/Alert®) culture system (Table 1). Except when it became positive during overnight culture, the blood culture fluid was taken immediately when found to be turbid or when alerted by the machine's alarm, and subjected to subculture and biochemical tests [3, 4] for species identification or directly to the MAb-LA [7] test. As well as these culture-positive specimens, there were 552 blood samples from patients suspected of having septicaemia which showed no bacterial or fungal growth after incubation for 7 days. All these culture-negative specimens were also subjected to the MAb-LA test.

MAb-LA test

The procedure for MAb-LA testing of the blood culture fluid was as described previously [7]. Briefly, the latex particles were coated with a pre-determined mixture of three MAbs specific for the 200-kDa surface antigen of *B. pseudomallei* [7, 9, 10]. The 1% sensitised latex suspension was mixed with an equal volume of the uncentrifuged positive blood culture fluid and the agglutination results were read within 1–2 min. The sensitivity of the test was *c.* $1–2 \times 10^6$ cfu/ml [7]. To minimise the occurrence of false positive results. 0.5 ml of all positive blood culture fluids was also subcultured into 2 ml of brain heart infusion (BHI) broth and incubated for 3 h before repeating the MAb-LA test.

Results

The 1369 culture-positive blood specimens used for this study were collected from patients admitted to 12 hospitals located all over the northeastern part of Thailand known to be an endemic region for melioidosis (Table 1). Of these, four were large regional hospitals equipped with automated blood-culture machines. With the exception of one (no. 5 in Table 1), the remaining seven hospitals were smaller provincial hospitals that used only manual culture systems. Of the 1369 culture-positive specimens, 204 were positive for *B. pseudomallei*. In these culture-positive specimens, the proportion of those positive for *B. pseudomallei* by the automated culture system was slightly lower than that with the manual culture system. However, this difference could be related to prevalence of melioidosis in different provinces. Details of the remaining 1165 specimens are shown in Table 2.

Among the 204 specimens culture positive for *B. pseudomallei*, 194 (95.1%) were positive by the MAb-LA test. When the blood culture fluid was used directly for testing, a total of 10 *B. pseudomallei* culture-positive specimens were negative in the MAb-LA test (five from each system). Six of these MAb-LA negative specimens became positive after subculturing in BHI. Of the remaining 1165 samples that were culture-positive for other organisms (Table 2), only three were positive by MAb-LA and all were from the manual culture systems. These three specimens yielded growth of *Acinetobacter baumanii* (1 of 16), *Enterobacter cloacae* (1 of 11) and *Escherichia coli* (1 of 225).

Table 2. MAb-LA test in blood culture fluids positive for *B. pseudomallei* and for other micro-organisms

Culture positive for	Samples tested	MAb-LA positive
B. pseudomallei	204	194*
Other micro-organisms		
Gram-positive bacteria	554†	0
Gram-negative bacteria	570	3‡
Mixed bacterial infections§	26	0
Fungi	15	0

*The number of positive samples rose to 200 after subculturing in BHI broth.
†Of these, 111 specimens were *Staphylococcus aureus*-positive, all of which were MAb-LA negative.
‡These three MAb-LA-positive specimens were culture-positive for *A. baumanii*, *E. cloacae* and *E. coli*.
§Two or three species were identified from the same blood culture broth.

Table 1. Sensitivity and specificity of the MAb-LA test of blood culture fluid from patients with community-acquired septicaemia

Hospital no.	Type of hospital	Culture system	MAb-LA positive/total culture positive for	
			B. pseudomallei	Others
1	Regional	Automated	34/36	0/160
2	Regional	Automated	46/46	0/239
3	Regional	Automated	7/8	0/241
4	Regional	Automated	27/29	0/184
5	Provincial	Automated	1/1	0/31
6	Provincial	Manual	18/22	0/49
7	Provincial	Manual	6/6	1/19
8	Provincial	Manual	1/1	0/7
9	Provincial	Manual	8/8	0/39
10	Provincial	Manual	13/14	0/97
11	Provincial	Manual	4/4	0/16
12	Provincial	Manual	29/29	2/83
Total			194/204	3/1165

Therefore, the specificity of the MAb-LA test was >99%. The overall performance of the MAb-LA test performed directly with positive blood culture fluids was acceptable, with 95.1% sensitivity, 99.7% specificity, 98.8% positive predictive value and 99.2% negative predictive value. The sensitivity figure could be raised to 98.1% if the MAb-LA test was performed with the BHI broth subcultured from the blood culture fluid and incubated for 3 h before repeating the MAb-LA test. Of the 552 culture-negative specimens (i.e., 'no growth' after incubation for a further 7 days at 37°C), only one was positive in the MAb-LA test. When these 552 specimens were included in the calculation, the figures for sensitivity, specificity, positive predictive and negative predictive values were not significantly different from those calculated from the culture-positive specimens alone.

Discussion

These data probably represent the largest prospective study of community-acquired septicaemia in an area endemic for melioidosis in any one rainy season. The overall percentage of *B. pseudomallei*-positive results among the 1369 positive cultures was *c*. 15%. Previously, Chaowagul *et al*. [1] found a higher prevalence (*c*. 30%) in a study 10 years earlier in hospital no. 1 of the current study. The proportion of *B. pseudomallei*-positive specimens in the present series for this hospital was 18% (Table 1). The overall performance of the MAb-LA test reported here was similar to the preliminary results reported previously by this group [7]. In that report, only two hospitals, both of which used an automated culture system (either BacT/Alert® or Bactec® system), were selected because at that time only preliminary data were required to see if a study with larger series of patients should be done. The present study expanded the number of clinical settings from two hospitals to 12 hospitals, some of which had no automated culture system and so the culture could only be done manually (Table 1). Although the latter procedure was less sensitive than the automated system (unpublished observations), the results of the MAb-LA test appeared to work just as well with the manual system employed by these seven provincial hospitals.

There is no straightforward explanation for the three specimens that gave false positive results by the MAb-LA test. True cross-reaction can be ruled out, as the MAb-LA reagent did not react with these three bacterial species (*A. baumanii, Ent. cloacae* and *E. coli*) when grown in ordinary bacterial culture. However, it is possible that the patients might have had mixed bacterial infection and that the small number of *B. pseudomallei* in the blood culture fluid was overlooked. Recently, Steinmetz *et al*. [11] reported the value of the MAb-based latex agglutination test for the identification of laboratory-grown *B. pseudomallei*. Their test was also based on the use of a MAb specific for the exopolysaccharide of *B. pseudomallei*. This group recently noted that their MAb appeared to react with a different epitope, which was most likely present in the same 200-kDa surface component that reacted with the MAbs used in the present study (unpublished observations). The data obtained in the present study showed that the MAb-LA test developed as described previously [7] is valuable not only in a laboratory setting, but also in clinical settings in rural areas where melioidosis predominantly occurs and facilities for automated blood culture systems are not usually available.

Similar results on specificity and sensitivity were reported recently by two other groups of investigators in a smaller scale study [12, 13]. Moreover, these investigators limited their studies to the blood culture fluids positive only for gram-negative bacteria. One group [12] employed a lipopolysaccharide (LPS)-specific MAb, which not only failed to react with virulent clinical isolates possessing atypical LPS component [14], but also reacted with avirulent arabinose-positive soil isolates [10]. Also in their study, the blood culture fluids has to be diluted first with a special diluent buffer to enhance the sensitivity of their latex agglutination test system. Therefore, their protocol required one additional step. The other group used a MAb reactive with a 30-kDa protein antigen, which also cross-reacted with some gram-positive bacteria [13]. Their test system gave only 86% specificity, which could be increased after either centrifugation or subculturing in BHI broth. This again added one additional step to their protocol to reach a specificity value approaching that reported in the current study. As the MAb used in the present system appeared to be more specific than those used by the other investigators and this MAb-LA test system was more simple to perform and worked equally well with both the manual and automated culture systems, it is recommended that this combined method be used to replace, or is used in conjunction with, the more time-consuming biochemical method for the diagnosis of septicaemic melioidosis. The method is well suited to diagnostic laboratories with minimal equipment and less experienced laboratory personnel, both in the endemic and non-endemic areas for *B. pseudomallei* infection. The method is economical, simple and highly reproducible, with specificity and sensitivity values approaching 100% for diagnosis of septicaemic melioidosis. The reagent is stable for >1 year when kept at 4°C (unpublished observations).

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