Short reports

Latex agglutination for rapid detection of *Pseudomonas pseudomallei* antigen in urine of patients with melioidosis

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Abstract

A latex agglutination test for the detection of *Pseudomonas pseudomallei* antigen in urine was evaluated for the rapid diagnosis of melioidosis. With unconcentrated urine, antigen was detected in only 18% of patients with melioidosis overall. However, when urine was concentrated 100-fold, antigen was detected in 47% overall and in 67% of patients with septicaemia or disseminated infection, in whom a rapid diagnosis is most important. The specificity of the test was 100%. These results compared favourably with an enzyme immunoassay. This latex agglutination test is a simple, rapid and highly specific method of diagnosing melioidosis, and will be particularly useful in areas with limited laboratory facilities.

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Melioidosis is a potentially fatal infection caused by the Gram negative bacillus *Pseudomonas pseudomallei*, an environmental saprophyte found in moist soil and water of many tropical countries. Melioidosis is endemic in South East Asia and northern Australia, and has a wide range of clinical presentations from mild chronic illness to fulminant infection. Although any organ may be involved, soft tissue and visceral abscesses, pneumonia and septicaemia are particularly common.¹ Melioidosis is a common cause of morbidity and mortality in the rural, rice farming, communities of northeast Thailand.² Confirmation of the diagnosis by culture requires special media and may take several days.³

Pseudomonas pseudomallei is unresponsive to those antibiotics (gentamicin plus a penicillin) commonly used to treat community acquired septicaemia. Therefore, a rapid presumptive diagnosis is essential so that patients may be given appropriate therapy, such as ceftazidime.⁴ Immunofluorescence microscopy to detect whole organisms⁵ and urinary antigen detection by enzyme linked immunosorbent assay (ELISA)⁶

have been applied to melioidosis but both techniques require expensive equipment, which is not widely available in these endemic areas. Therefore, we have evaluated a simple and rapid latex agglutination test⁷ for the detection of *Pseudomonas pseudomallei* antigen in urine of patients with melioidosis and compared it with the ELISA.

Methods

All patients were admitted to Sappasitprasong Hospital, Ubon Ratchatani, between August 1991 and July 1993. Melioidosis was diagnosed definitively by the isolation of *Pseudomonas pseudomallei* from clinical specimens as described previously.³ Urine specimens were collected on the day of admission from 100 patients with culture positive melioidosis. Of these, 14 had disseminated infection (septicaemia with two or more foci of infection), 40 had septicaemia with one or no focus of infection. Forty five patients had localised *Pseudomonas pseudomallei* infections including pneumonia or lung abscesses (n=23), liver or splenic abscesses (n=7), parotid abscesses (n=5), soft tissue and other sites (n=10). The single remaining patient had a localised urinary tract infection ($\geq 10^5$ colony forming units per ml (cfu/ml)). The mean duration of symptoms before entering the study was 17 days (median 10 days, range two to 100 days).

Urine samples were also obtained from 200 hospitalised patients with a variety of diagnoses other than melioidosis (64 of these had bacteruria $\geq 10^5$ cfu/ml caused by organisms other than *Pseudomonas pseudomallei*). This control group of patients came from the same rice farming communities as those with melioidosis.

PREPARATION OF THE LATEX TEST

The latex agglutination test was prepared as described previously.⁷ Briefly, a rabbit polyclonal antibody produced against whole boiled cells of a reference strain of *Pseudomonas pseudomallei* (NCTC 1688) was used to coat the test latex particles. Control latex particles were coated with normal rabbit serum. Suspensions

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Comparison of latex agglutination and ELISA for detection of *P. pseudomallei* antigen in urine, categorised by site of infection

Site of infection	Latex agglutination test		ELISA
	Unconcentrated urine n (%)	Concentrated urine ($\times 100$) n (%)	(≥ 1 in 10 dilution of urine) n (%)
Disseminated*	6/14 (43)	11/14 (79)	10/10 (100)
Septicaemic	7/40 (17.5)	23/37 (62)	26/33 (79)
Pulmonary infection	3/23 (13)	6/22 (27)	11/21 (52)
Other localised infections	1/22 (4.5)	3/19 (16)	11/17 (65)
Urinary tract infection**	1/1 (100)	1/1 (100)	1/1 (100)
All patients	18/100 (18)	44/93 (47)	58/82 (71)

*Disseminated = septicaemia and two or more focal sites of infection.

**Urinary tract infection = *P. pseudomallei* $>10^5$ cfu/ml urine.

were stored at 4°C for up to one year and brought to room temperature before use.

LATEX AGGLUTINATION METHOD

All urine samples were boiled for five minutes to eliminate non-specific reactions, cooled and centrifuged (3000 rpm for five minutes) before testing the supernatant fluid. Urine (40 μ l) was mixed with 20 μ l latex suspension on a white card. After spreading, the card was rocked gently for three minutes. Agglutination with the test latex but not the control latex indicated a positive result.

Urine samples with sufficient volume (≥ 5 ml) were then concentrated 100-fold in a Minicon B15 concentrator (Amicon Ltd, Stonehouse, UK) before retesting with the latex suspension. This takes approximately 60 to 90 minutes.

ELISA METHOD

The antigen detection ELISA was performed as described previously.⁶ The ELISA utilises a polyclonal anti-*P. pseudomallei* antibody conjugated to fluorescein isothiocyanate (FITC) and an anti-FITC antibody amplification system. Urine samples were stored at -30°C and tested in batches. Boiled and centrifuged urine was tested at a range of dilutions.

Results

A total of 100 unconcentrated test urine samples and 200 control urine samples were analysed. Subsequently, 93 test samples and 40 control samples were concentrated 100-fold and retested. The results were categorised according to the site of infection (table). The overall sensitivity was 18% for unconcentrated urine but increased to 47% for concentrated urine samples. Antigen detection was highest in patients with disseminated or septicaemic infection (34 of 51; 67%) compared with localised disease (10 of 42; 22%). There were no positive reactions with the 200 control urine samples, including the 40 concentrated samples, giving a specificity of 100% for the test.

Eighty two test urine samples were available for antigen assay by ELISA. Using the previously recommended cut off dilution of 1 in 10 to ensure high specificity, the overall sensitivity for this group of patients with melioidosis

was 71%. Again, the sensitivity was higher in those patients with disseminated infection or septicaemia (36 of 43; 84%) compared with those with localised infections (23 of 39; 58%).

Discussion

Severe melioidosis has an extremely high mortality if appropriate treatment is not given promptly.¹ The current antibiotic of choice, ceftazidime,⁴ is expensive and is not used as first line empirical therapy for community acquired septicaemia. Therefore, rapid tests are required to confirm diagnosis. If suitable specimens such as sputum or pus are available for examination, immunofluorescence microscopy is extremely useful.⁵ Urine is readily available from almost all patients, including children from whom other specimens may be difficult to obtain. The rapid diagnosis of melioidosis by detection of urinary antigen has obvious advantages. An enzyme immunoassay has been developed,⁶ but requires expensive equipment and is most suitable for batch testing.

Latex agglutination tests are used widely for the detection of bacterial antigens in cerebrospinal fluid. They have also been applied to the detection of antigen in urine, with variable results, although sensitivity of the test often improves after concentration of urine. In one study, using urine concentrated 25-fold, *Haemophilus influenzae* type b antigen was detected in urine in 20 of 23 children with culture positive meningitis or septicaemia.⁸ In a study of pneumococcal pneumonia in Gambian children O'Neill *et al.*⁹ used serotype specific latex tests to detect urinary antigen in 50% of culture positive cases. The sensitivity of the test increased to 76% after concentrating the urine 25-fold.

In this study the latex agglutination test, using unconcentrated urine, could detect *P. pseudomallei* antigen only in 18% of all patients with melioidosis. This increased to 43% in the group of patients with disseminated disease, presumably reflecting the greater bacterial load and thus higher concentrations of antigen excreted in the urine. Concentrating urine 100-fold significantly improved the sensitivity of the test. The increase in sensitivity occurred in patients with localised forms of melioidosis as well as in those with septicaemia. Antigen was still only detected in 47% patients overall, but increased to over 60% in patients with septicaemia and nearly 80% in those with disseminated infection. Therefore, this test will be most useful in those patients with life threatening disease in whom rapid diagnosis and appropriate antibiotic treatment is urgently needed. In the present study the ELISA was considerably better at detecting urinary antigen, although the specificity (96%) published previously⁶ is lower than the 100% specificity for the latex test. The ELISA is also unsuitable for immediate testing of patients on admission to hospital.

This latex agglutination test offers a simple, cheap, rapid method of diagnosing melioidosis. A correct diagnosis may be achieved, within two hours, for two thirds of patients with severe

melioidosis or septicaemia. It will be particularly useful in laboratories with limited facilities. The cost of concentrating one urine sample 100-fold is approximately £3, although it may be possible to increase the sensitivity of the test by other, cheaper methods¹⁰ making it more widely applicable.

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