

A DECADE OF EXPERIENCE OF THE UNITED KINGDOM'S MELIOIDOSIS DIAGNOSTIC SERVICE



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ABSTRACT

Melioidosis is rare in the Europe and is invariably associated with travel to tropical areas, particularly Southeast Asia. Over the last decade 49 patient isolates were confirmed as *Burkholderia pseudomallei*, and most cases were from the UK (33) following visits to Thailand, Bangladesh and Australia but 3 were cystic fibrosis patients who had acquired the infection in Malaysia, British Virgin Islands and Brazil. Isolates were also received for confirmation from European laboratories. In addition we received 21 isolates misidentified as *B. pseudomallei* by diagnostic laboratories. Isolates are sub-cultured on Ashdown's agar and blood agar and screened for resistance to 10µg gentamicin and 10µg colistin discs. Before 2004, identification was confirmed by API 20NE supplemented with selected ammonium salt based sugar tests. Isolates giving equivocal results are further tested for agglutination with an anti-lipopolysaccharide (LPS) antibody latex reagent. In the last 3 years we have screened isolates by cellular fatty acid analysis by gas chromatography (MIDI, Sherlock) for 2-hydroxymyristic acid; 14:0 2OH which allows confirmation within 24 h. Isolates are further characterised by ribotype and PFGE for epidemiological studies.

We perform an in-house ELISA for the determination of serum antibodies to the conserved LPS antigen of *B. pseudomallei*. Data from the last 3 years show that 202 serum samples from 170 patients were tested. Eleven gave positive titres (≥ 2000) and 8 patients were culture positive for *B. pseudomallei*. Further investigation by immunoblotting of serum from 1 patient revealed variation in LPS epitopes of the infecting strain of *B. pseudomallei*.

INTRODUCTION

We have provided a laboratory diagnostic and clinical advice service to support the diagnosis of melioidosis in the United Kingdom for several years. The laboratory is based at the Centre for Infections, Colindale, London and clinical advice is given by three medical microbiologists all of whom worked for periods at the Wellcome-Mahidol Unit in Bangkok, Thailand. The service was started in 1988 and between then and 1998 we were consulted about 15 cases of melioidosis. The most common country of origin was Thailand (5) and Bangladesh (5) but others were from Malaysia (2), Pakistan (1), India (1) and Indonesia (1). Six cases occurred in 1998 and three of these were associated with Bangladeshi immigrants returning to the UK following visits to the heavily flooded Sylhet district (Dance *et al.* 1999).

We present here our experience of the last 10 years providing reference microbiology and clinical support for melioidosis.

METHODS

When clinicians contact the laboratory regarding suspected cases of melioidosis, in addition to submitting appropriate specimens, they are put in contact with one of the medical microbiologists to review the patient's history and to discuss appropriate investigations and clinical and public health management.

Bacterial isolates recovered from suspected cases are received at Colindale and sub-cultured on Ashdown's agar with colistin and gentamicin (10 µg) discs (Figure 1). The plates are examined after 48h incubation for colonial morphology. Isolates are also tested in the API20NE incubated at 30°C and read after 48 h. Fatty acids are extracted by the protocol outlined in Figure 2 and analyzed by gas liquid chromatography (MIDI, Sherlock). A DNA extract is prepared from a boiled cell suspension and subjected to 16S rDNA sequencing following by BLAST analysis against the GenBank database. For selected isolates, lipopolysaccharide (LPS) is prepared by hot phenol-water or protease K digestion for immunoblot analysis.

For serodiagnostic tests, plates are coated with an LPS- rich extract of *B. pseudomallei* strain 204 and antibody binding is detected in a standard ELISA.

Figure 1. Screening for *B. pseudomallei*: resistance to gentamicin and colistin of 24 h growth of isolate on blood agar

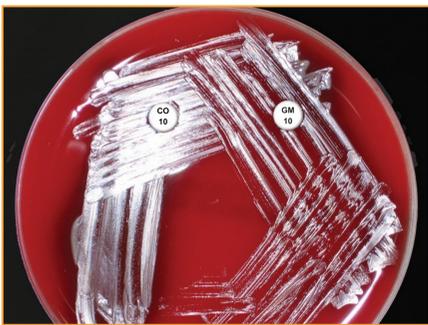
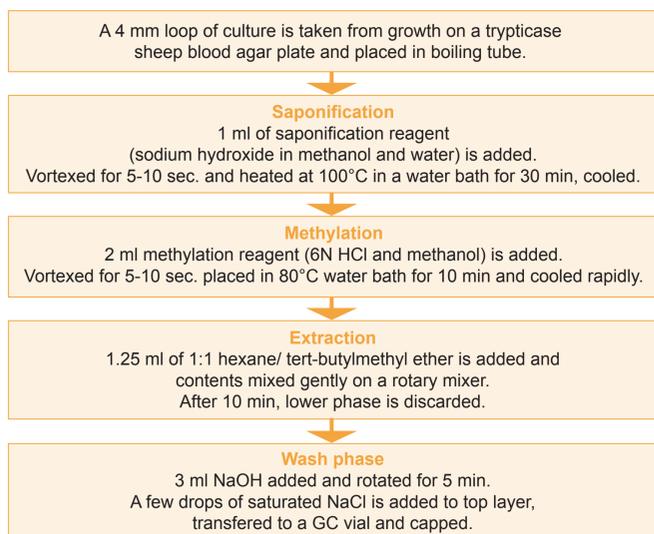


Figure 2. Protocol for cellular fatty acid extraction



RESULTS

IDENTIFICATION

Table 1 shows the origin of 33 confirmed isolates of *B. pseudomallei* from individual patients received by the service. Twenty-two patients were referred from UK hospitals commonly with a history of travel in South East Asia or the Indian subcontinent (Thailand 9, Bangladesh 6, India 2, Malaysia 2, Vietnam 2). The remaining isolates were referred from a range of other countries, predominantly in Europe but also in Asia and South America. Four patients had cystic fibrosis (CF) and had initially been thought to harbour *B. cepacia* complex organisms in the respiratory tract. Isolates were recovered from various body sites with blood and sputum predominating.

Most isolates gave typical colonial morphologies (Figure 3) and were identified by API20NE, the majority giving 'excellent identification'; code 1156577 was the most common. Variation in assimilation of adipic acid, phenylacetic acid and trisodium citrate along with arginine dihydrolase tests was observed and this sometimes resulted in 'low discrimination' or 'doubtful profile.'

Confirmation of *B. pseudomallei* was provided by the presence of 2-hydroxymyristic acid; 14:0 2OH (Figure 4).

Table 1. Origin of isolates of *B. pseudomallei* 1997-2007

Year	Referred from	Travel history	Body site	Number
1997	UK	Thailand	Blood	SID2015
1998	UK	Bangladesh	Sputum/blood ⁴	SID2889
1998	UK	Thailand	Tissue	SID3319
1998	Italy	Thailand	Sputum/CF	SID3477
1998	UK	Not given	Abscess	SID3511
1999	Sweden	Vietnam	Blood	SID3584
1999	UK	Malaysia	Sputum/CF	SID3783
1999	UK	Bangladesh	Blood/bone	SID3811
1999	Netherlands	Thailand	Sputum	SID4075 ¹
1999	Netherlands	Thailand	Cervix	SID4152 ¹
1999	UK	Malaysia	Blood	SID4349
1999	UK	Bangladesh	Bone	SID4717
2000	UK	Thailand	Wound	SID5278
2000	Sweden	Not given	Not given	SID5311
2001	UK	Thailand	Blood/sputum	SID5752
2001	UK	India	Aspirate parotid	SID7161
2003	Brunei	Brunei	Blood	SID1131
2003	Netherlands	Brazil	Blood	SID1615
2003	UK	Thailand	Blood	SID1872
2003	UK	Bangladesh	Blood	H034580128
2004	UK	Thailand	Sputum	H041980220
2004	UK	Bangladesh	Lymph node	H043740683
2005	Sweden	Vietnam	Blood	H044200452
2005	UK	Thailand	Blood	H051600635
2005	UK	Thailand/Vietnam	Sputum (CF)	H054100490
2006	UK	India	Pus (abscess)	H054640145
2006	UK	Thailand	Abscess	H060960487
2006	UK	Bangladesh	Blood/sputum	H061220286
2006	UK	Thailand	Lung nodules	H061320428
2006	Brazil	Brazil	Sputum (CF)	H061740680
2006	UK	Thailand	Blood/urine	H062400314
2007	UK	Virgin Islands	Sputum	H064560522

¹Abbink *et al.* (2001).

Figure 3. Growth of *B. pseudomallei* on Ashdowns agar. A) typical colony, B) mucoid colony

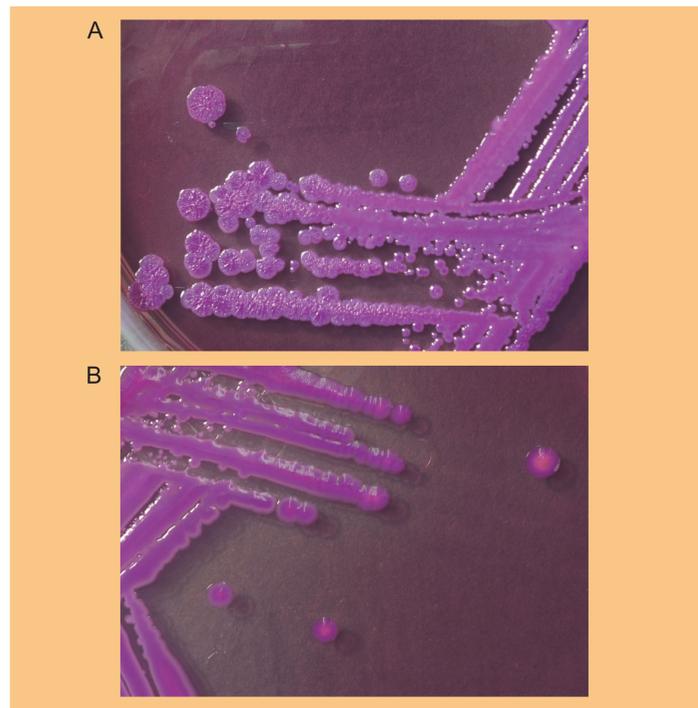
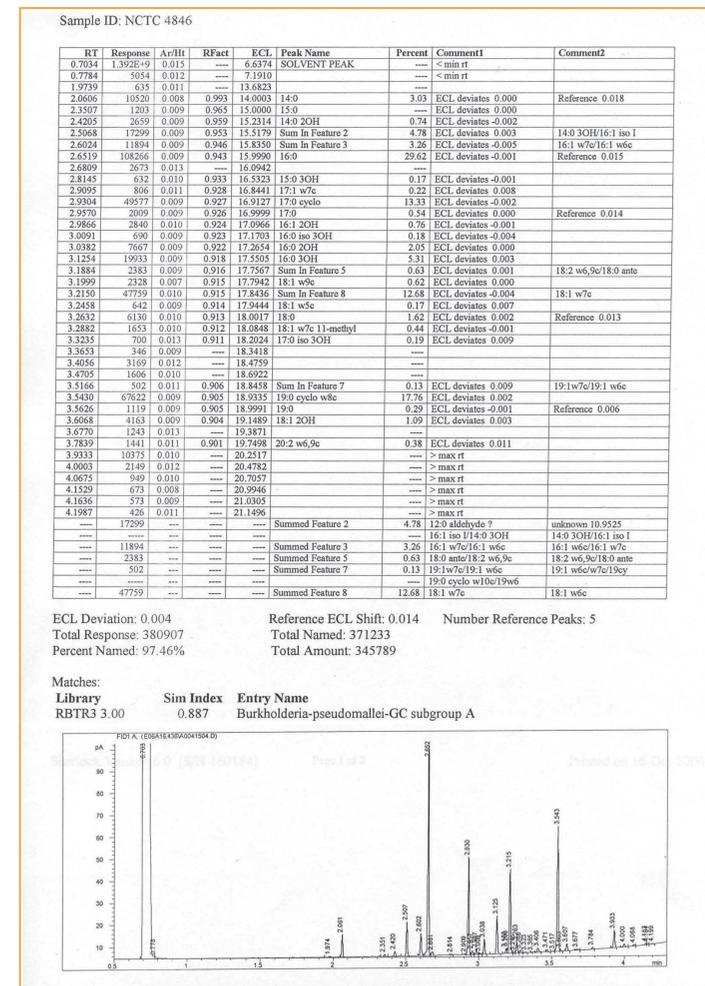
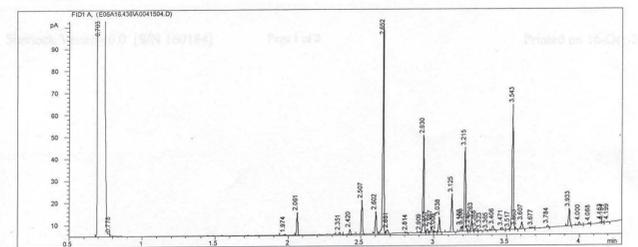


Figure 4. Fatty acid profile of strain of *B. pseudomallei*



ECL Deviation: 0.004 Reference ECL Shift: 0.014 Number Reference Peaks: 5
Total Response: 380907 Total Named: 371233
Percent Named: 97.46% Total Amount: 345789

Matches:
Library: RBTR3 3.00 Sim Index: 0.887 Entry Name: Burkholderia-pseudomallei-GC subgroup A



SERODIAGNOSIS

Two hundred and ninety-eight serum samples were received and 27 (9.1%) gave titres equal to or greater than the positive control serum (≥2000) in ELISA; of these 19 were known to be culture positive. A number of sera were submitted from casualties of the tsunami of 2004, particularly from Europe but none showed antibodies to *B. pseudomallei*. The conservation of the LPS antigen of reference strain 204 allowed the identification of serum antibodies in all but one seropositive patient. This patient's serum was negative in ELISA with strain 204 but gave a titre of 4000 with their own strain. Comparison of LPS antigens from the two strains by SDS-PAGE and immunoblotting showed that they differed in O-repeating units (Brent *et al.* 2007, Figure 5).

Serum samples are sometimes submitted from laboratory or nursing staff who have had accidental exposure to the organism (at containment level 2) or to body fluids from patients. None have shown evidence of seroconversion.

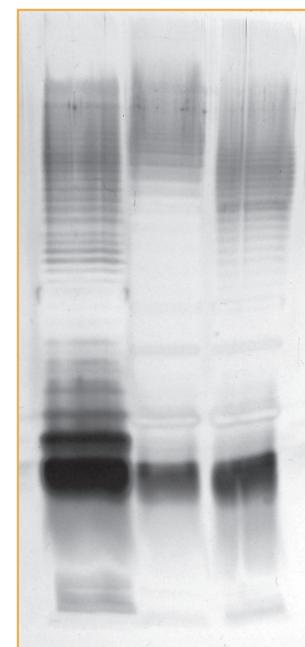


Figure 5. SDS-PAGE of LPS from patient's strain and strain 204 showing different O-repeating units (Courtesy of A. Weisner)

REFERENCES

Abbink FC, Orendi JM, de Beaufort AJ. Mother-to-child transmission of *Burkholderia pseudomallei*. N Engl J Med 2001;344:1171.
Brent AJ, Matthews PC, Dance DAB, Pitt TL, Handy R. Misdiagnosing melioidosis. Emerg Infect Dis 2007;13:349.
Dance DAB, Smith MD, Aucken HM, Pitt TL. Imported melioidosis in England and Wales. Lancet 1999;353:208.