

## CLINICAL REVIEW

### Melioidosis: a review

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YC Foong<sup>1</sup>, M Tan<sup>1</sup>, RS Bradbury<sup>2</sup>

<sup>1</sup>*School of Medicine, University of Tasmania, Hobart, Tasmania, Australia*

<sup>2</sup>*School of Medical and Applied Sciences, Central Queensland University, North Rockhampton, Queensland, Australia.*

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**Foong YC, Tan M, Bradbury RS**

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## ABSTRACT

The disease melioidosis, caused by the bacterium *Burkholderia pseudomallei*, remains an important and sometimes neglected cause of disease in tropical regions of Australia. Infection may present in myriad ways, and diagnosis often requires consideration of this organism prior to culture. Laboratory identification of *B. pseudomallei* requires specialised testing beyond that available in many routine diagnostic microbiology laboratories. For this reason, cases outside of the traditional endemic zone, often occurring years after initial exposure to the organism, may remain undiagnosed or are delayed in diagnosis. Furthermore, the high levels of intrinsic antimicrobial resistance associated with *B. pseudomallei* often render empirical therapies ineffective. Health professionals, particularly those in rural and remote areas of Australia, must consider melioidosis in their differential diagnoses and remain abreast of advances in the field of this important emerging disease.

**Key words:** Burkholderia, melioidosis, pseudomallei, review.

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## Epidemiology

Melioidosis is an emerging infectious disease that is endemic in South-East Asia and the 'Top End' of Australia. Melioidosis represents the most common cause of fatal community-acquired septicaemia and pneumonia in parts of north-eastern

Thailand as well as at the Royal Darwin Hospital<sup>1,2</sup>; these areas are considered hyper-endemic for melioidosis. However, emerging data indicate that the geographic distribution of melioidosis may be far wider than previously suspected<sup>3</sup>. There is evidence suggesting it is now endemic in the Indian subcontinent, southern China, Hong Kong and Taiwan, as well as reports of sporadic cases in Africa, the



Americas, the Middle East and island communities in the Pacific and Indian oceans<sup>3</sup>. Low levels of sporadic infection, with peaks at times of high rainfall, are seen in endemic areas. Outbreaks and case clusters occur but are rare. Occasional foci of infection have occurred in subtropical and temperate regions, such as the one human and several veterinary cases occurring near Perth, Western Australia, in the early 1990s<sup>4</sup> and a case report from south-eastern Queensland<sup>5</sup>. An outbreak of human and veterinary cases of melioidosis, associated with zoos and equestrian clubs, was recorded in France during the 1970s<sup>6</sup>. It remains uncertain whether the occurrence of melioidosis has increased in recent years, or whether it was simply previously under-diagnosed and under-recognised by clinicians.

Studies conducted between 1989 and 2003 have shown an increasing incidence of melioidosis in the Top End of Australia<sup>7,8</sup>. This compares with an increase from 4.4 per 100 000 (1987–1991) to 21.3 per 100 000 in 2006 in Ubon Ratchatani province, Thailand<sup>9</sup>. Rainfall in particular seems to have an important role in the disease: incidence rose from 16.5 to 34.5 cases per 100 000 people in the Top End over the very heavy 1997–1998 wet season, and severity of the disease increased significantly<sup>9,10</sup>. Most recently, incidence reached record rates (50.2 cases per 100 000 people) over the 2009–2010 wet season, which was again associated with heavy rainfall<sup>11</sup>.

## Environment

Melioidosis is caused by the bacterium *Burkholderia pseudomallei*, a soil and freshwater saprophyte. It was first discovered by Alfred Whitmore and C.S. Krishnaswami amongst morphine addicts in Burma in 1911<sup>12,13</sup>. Although initially classified in the genus *Pseudomonas*, it was reclassified under a new genus, *Burkholderia*, in 1992. The organism has been recovered from wet soils, streams, pools, stagnant water and rice paddy fields in particular. The demonstration of *B. pseudomallei* survival within spores of mycorrhizal fungi<sup>14</sup> and environmental amoebae<sup>15</sup> has led to the proposal that these soil organisms may represent a niche for survival and dissemination in the natural environment.

The commonest mode of transmission is via direct inoculation of contaminated soil and surface water through skin abrasions. Human-to-human transmission, transmission through inhalation of polluted water and contact with contaminated ground water have been reported<sup>16–18</sup>. The disease affects a wide range of susceptible animals, including cats, goats, sheep and horses<sup>19,20</sup>. Zoonotic transmission is reported to be rare, with only three possible cases being reported in Australia<sup>19</sup>.

## Risk factors

The prevalence of melioidosis is highest amongst immunocompromised individuals and those with significant comorbidities. Diabetes mellitus is the most important host risk factor for the disease<sup>21,22</sup>, with one study finding 60.9% of affected patients had diabetes mellitus<sup>21</sup>. Other high-risk populations include individuals with chronic renal impairment (present in 12% of cases), pulmonary disease (27% of cases)<sup>2</sup>, thalassaemia<sup>22</sup>, congestive heart failure, corticosteroid therapy, malignancy (particularly leukaemia and lymphoma) and immunosuppression<sup>20</sup>. It is significantly associated with prolonged alcohol intake, with a history of alcohol abuse evident in 39% of cases in Australia's Northern Territory<sup>20</sup>; and in 12% of melioidosis cases in Thailand<sup>21</sup>. Ingestion of kava (a plant-based relaxant) has also been linked to an increased risk of contracting melioidosis, with 8% of cases in Northern Australia attributed to high rates of kava consumption<sup>2</sup>. The first four cases of melioidosis reported in New Caledonia occurred between 1999 and 2004, and interestingly all four individuals were heavy consumers of kava<sup>22</sup>.

Continual occupational exposure to soils and ground water contributes to an increased risk of contracting melioidosis, placing rice farmers and labourers at greater risk<sup>23</sup>. Additionally, infection can also occur indirectly through contamination of generally harmless materials. A case in the Northern Territory reported two mechanics infected with *B. pseudomallei* from contaminated handwashing detergent, due to the introduction of a hose that had been contaminated by nearby soil<sup>24</sup>. Similar cases have been reported of melioidosis from contaminated chlorhexidine antiseptic wash<sup>24</sup>.



Melioidosis can also occur in healthy and low-risk individuals<sup>25</sup>. While it is most prevalent in those in the fourth and fifth decades of life<sup>26</sup>, the disease can affect persons of all ages including children<sup>27</sup>. A Thai study found that 36% of melioidosis cases studied occurred in otherwise healthy, low-risk patients<sup>28</sup>. A study conducted in the Northern Territory found that as many as 20% of symptomatic hosts were considered to be at low risk of the disease; one death was reported<sup>2</sup>. Individuals with no risk-factors experience less-severe symptoms and fatalities are rare<sup>25</sup>.

If patients present to their health practitioner with a history of recent travel or residence in a *B. pseudomallei* endemic region – particularly in the wet season; and if they possess predisposing host-factors such as diabetes mellitus, chronic renal failure or a history of alcohol abuse – melioidosis must be considered. A low clinical threshold for a diagnosis of melioidosis in such patients will result in early treatment and mitigation of disease severity<sup>25</sup>.

## Clinical features

There are a variety of classifications for the clinical presentations of melioidosis<sup>29-31</sup>. Melioidosis is divided into acute, subacute and chronic cases as suggested by Howe et al<sup>29</sup>. Another classification method divided the disease presentations into disseminated septicaemic melioidosis, non-disseminated septicaemic melioidosis, localised melioidosis, transient bacteremia, probable and subclinical melioidosis<sup>30</sup>. Severe melioidosis may present as a disseminated septicaemic, non-disseminated septicaemic or localised infection. The wide range of possible presentations often means that melioidosis represents a diagnostic challenge, particularly for those who may not encounter the disease on a frequent basis.

### Subclinical

Most people exposed to the bacteria do not have any clinical symptoms. In endemic areas seroconversion occurs once children are exposed to wet soil (about 25% seroconvert

annually between 6 months and 4 years), resulting in high rates of seropositivity<sup>32,33</sup>. However the relevance of this in terms of a future clinical disease due to a latent focus is still quite unclear, although Currie et al. state that most cases are a result of recent acquisition, not re-activation<sup>34</sup>.

### Acute

Acute cases are those where symptoms were present for less than 2 months. In most patients, mean incubation was 9 days (range 1–21 days), although cases up to 62 years after initial exposure have been reported<sup>35</sup>. The spectrum of clinical presentations range from the severe, fulminant disease (such as multi-organ abscesses) to asymptomatic or minor localised infections. The type of presentation may be influenced by the magnitude of exposure, mode of acquisition, host factors and risk factors. Melioidosis may present as pneumonia, skin abscesses, internal organ abscess, osteomyelitis, septic arthritis and even encephalomyelitis<sup>2</sup>. Almost any organ system may be affected, including the lungs, kidneys, prostate, skin, liver, parotids and the brain<sup>20,36,37</sup>. In the Australian context, acute melioidosis accounts for a large majority of presentations (up to 91% compared to 9% for chronic disease)<sup>38</sup>. It is interesting to note that *B. pseudomallei* prostatic abscesses (Fig1), which had been thought of as uncommon, have been shown to be prevalent in Australian patients<sup>37,39</sup>. This range of unusual pathologies due to melioidosis demonstrates the need to perform extensive investigations in affected patients.

Pneumonia is the most common presentation of melioidosis, accounting for roughly half of all cases (Fig2). This finding is consistent across a wide variety of studies in different areas<sup>2,20,38,40-43</sup>, and is more commonly due to haematogenous dissemination to the lungs rather than inhalation of the pathogen<sup>44</sup>. The severity of the condition varies widely, from septic shock to mild undifferentiated pneumonia. Septic shock patients may present acutely unwell with high fevers and only slight cough or pleuritic pain, whereas patients with undifferentiated pneumonia will present with a more predominant cough accompanied by sputum and dyspnoea<sup>44</sup>. Radiography may range from diffuse nodular infiltrates in



both lungs of septic shock patients to discrete and progressive consolidation in one or more lobes for those with undifferentiated pneumonia. It is most commonly found in the upper lobes, and pleural effusions and empyema may occur, particularly with lower lobe disease<sup>41,45</sup>. Melioidosis may be mistaken for tuberculosis and thus a high index of suspicion is required, particularly when encountering such cases in endemic areas. *B. pseudomallei* pneumonia is often associated with disseminated disease, causing septic shock and multi-organ dysfunction.

## Chronic

Chronic melioidosis is defined as an illness where symptoms have lasted for longer than 2 months at presentation<sup>2</sup>. Patients present with a chronic respiratory illness that mimics tuberculosis, displaying signs such as fevers, weight loss and a productive cough with or without haemoptysis. There are often long-standing abscesses involving multiple sites as well.

## Latency

At one point, latent melioidosis was referred to as the 'Vietnam time bomb'<sup>46</sup>, alluding to its potential to remain latent for long periods of time in returned servicemen from Vietnam<sup>28</sup>. Delays from primary exposure to active disease of up to 62 years have previously been reported<sup>35</sup>. This long period in between acquisition and clinical presentation may be due to a decrease in host immunocompetence (particularly with comorbidities such as diabetes mellitus, renal failure and alcoholism). Therefore, melioidosis cannot be excluded as a potential diagnosis in those who have not travelled to endemic areas for many years.

## Biological warfare

*B. pseudomallei* has been labelled a category B priority pathogen by the United States Centers for Disease Control and Prevention (CDC). The organism has been the source of international concern in the past, as a source of both interest

and concern as a 'potential bioterror agent'. Its properties have been investigated by the USA and potentially Iran, Iraq, North Korea and Syria<sup>28</sup>. Unlike most other bacteria, *B. pseudomallei* is readily available in the environment; it is simple and cost-effective to produce<sup>28</sup> with considerable potential for infectivity and transmission, particularly via inhalation of aerosolised bacteria<sup>20</sup>; and it is resistant to standard antibiotic treatments for community-acquired pneumonia. Furthermore, at present *B. pseudomallei* lacks an effective vaccine<sup>47</sup>. This, combined with its capacity to cause severe disease if a bacteraemia ensues, allows it a significant potential for high rates of morbidity and mortality.

Despite these properties and international concern, *B. pseudomallei* has never yet been utilised in biological warfare<sup>48</sup>, and the effectiveness of the bacteria as such an agent is not fully understood<sup>46</sup>. Whilst release amongst healthy military troops without underlying chronic disease may not result in mass casualties, those clinical cases that did occur, and the potential for latent disease in exposed soldiers, would affect morale. Release into a civilian population would result in higher casualties and potentially greater effects on morale.

## Diagnostic investigations

A diagnosis of melioidosis can be a very difficult, particularly when it occurs outside of endemic regions. The definitive diagnosis is made when *B. pseudomallei* is recovered from any site – it is never normal flora. Complete screening of patients is recommended (blood, sputum, urine, pus culture and throat swab)<sup>49-52</sup>, and a frequent oversight is to only order specimens from the affected site. Imaging, though not diagnostic of the disease, is often very useful to ascertain the extent of the disease. CT scans or ultrasound of the abdomen is recommended routinely to check for subclinical abscesses, particularly of the prostate, which is an area with a high incidence of abscesses in northern Australia<sup>52,53</sup>.

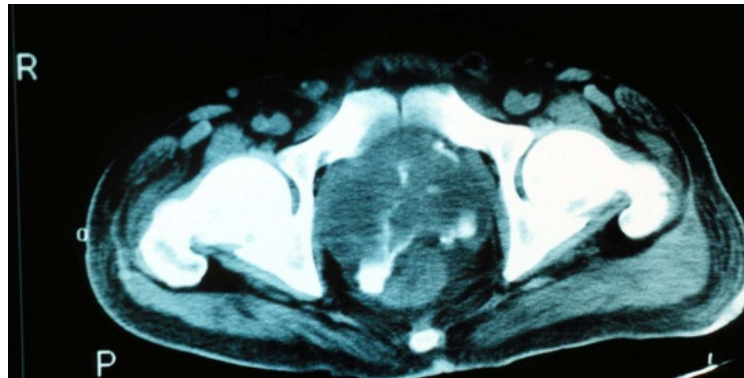


Figure 1: MRI showing *Burkholderia pseudomallei* prostatic abscess (courtesy Dr Robert Norton, Director of Pathology, Townsville Hospital).

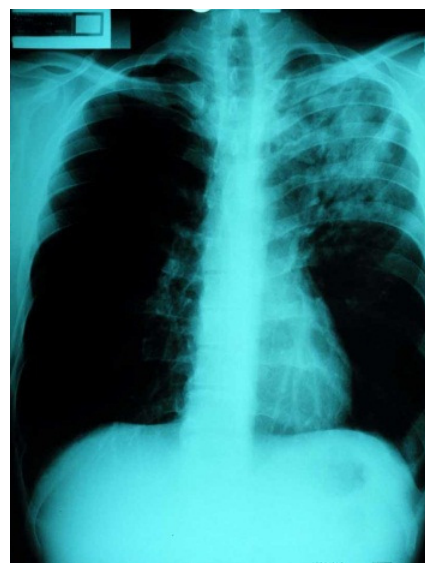
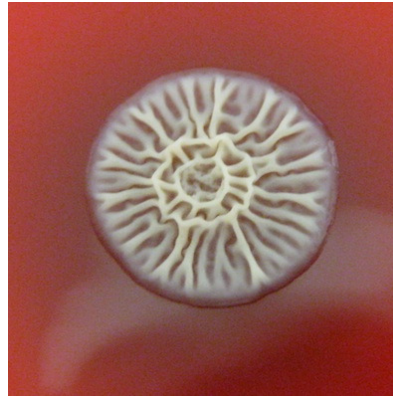


Figure 2: Chest X-ray showing left upper lobe pneumonia caused by *Burkholderia pseudomallei* (courtesy Dr Robert Norton, Director of Pathology, Townsville Hospital).

## Laboratory diagnosis

*B. pseudomallei* is an aerobic, oxidase positive, Gram negative bacillus. A vacuole in the centre of the cell leads to a 'safety pin' appearance in Gram stain<sup>54</sup>. The organism will grow on non-selective agar, most commonly forming small, smooth colonies with a metallic sheen and strong soil smell after 24

to 48 hours. After 3–5 days, colonies become dry and wrinkled (Fig3), resembling *Pseudomonas stutzeri*. Isolates are generally resistant to colistin and gentamicin<sup>54</sup>, but sensitive to amoxicillin/clavulanate<sup>55</sup>. Mucoïd phenotype and gentamicin sensitive strains have been previously identified<sup>54</sup>. *B. pseudomallei* is a biosafety level 3 pathogen, requiring specialised laboratory handling<sup>26</sup>.



**Figure 3:** *Burkholderia pseudomallei* colonies grown on blood agar after several days incubation; note the distinctive 'crinkled' colonial morphology (image from <http://micrognome.priobe.net>, reproduced with permission of Professor Tim Inglis).

## Selective media

The most commonly employed selective agar for *B. pseudomallei* is Ashdown's medium. The primary selective is gentamicin<sup>56</sup>, excluding the growth of rare gentamicin sensitive strains of *B. pseudomallei*<sup>56</sup>. Colonies on Ashdown's medium will grow within 72 h incubation in air at 37°C (with the exception of ceftazidime resistant mutants), and have a purple colour due to the neutral red indicator (Fig4). Ashdown's selective broth with colistin may be used to enhance the recovery of *B. pseudomallei* from throat and other non-sterile site specimens<sup>57</sup>.

Francis medium, a modification of Ashdown's medium, with an increase in gentamicin concentration from 4 mg/L to 8 mg/L and the neutral red indicator replaced with bromocresol purple, has been described<sup>58</sup>. The bromocresol purple indicator in Francis medium results in yellow-coloured colonies of *B. pseudomallei*. Gentamicin resistant *Klebsiella* and *Escherichia coli* colonies will also produce yellow colonies on this medium, but their negative oxidase reaction should easily exclude these from further investigation. A comparative study of Francis and Ashdown's medium using spiked sputum samples found a slightly improved sensitivity for Francis medium (76.7% vs 73.3%); 95% comparative confidence intervals for this study were not published<sup>58</sup>.

Laboratories outside of endemic areas that do not stock Ashdown's medium may use *Burkholderia cepacia* selective agar (BCSA) or *Pseudomonas* selective agar (PSA) as an alternative when specimens for *B. pseudomallei* investigation are occasionally submitted. One comparative study found that 70% of *B. pseudomallei* strains (originally recovered on Ashdown's agar) grew on BCSA, whilst 95% were detected on PSA<sup>59</sup>. Growth of *Pseudomonas aeruginosa* limits the utility of PSA as a selective agar for environmental samples or human specimens containing this organism. A separate study comparing Ashdown's agar, BCSA and *B. pseudomallei* selective agar (BPSA) found no significant difference in sensitivity for *B. pseudomallei* in all three media<sup>60</sup>.

## Phenotypic identification

The laboratory identification of *B. pseudomallei* remains problematic, particularly in laboratories without regular exposure to this organism. Isolation of an organism with colonial morphology resembling *B. pseudomallei* from a patient with relevant clinical notes should elicit suspicion, regardless of the length of time since that patient has visited an endemic area. The strong soil smell of *B. pseudomallei* colonies was a historically used method of screening suspicious oxidase positive colonies, but occupational health and safety considerations disallow this.



**Figure 4: *Burkholderia pseudomallei* colonies grown on Ashdown's agar after 2 days incubation (courtesy Dr Robert Norton, Director of Pathology, Townsville Hospital).**

Biochemical profile alone is insufficient to definitively identify an unknown oxidase positive Gram negative bacillus as *B. pseudomallei*. The most common biotypes for *B. pseudomallei* yielded by the API 20NE identification system are 1156576 and 1156577, a small number (7%) differing from these biotypes only in negative results for aesculin hydrolysis<sup>61</sup>. The Vitek 2 and WalkAway 96 systems showed respective sensitivities of 69% and 96% for the identification of *B. pseudomallei* with low discrimination identifications by the Vitek 2 common<sup>62</sup>. Whilst the API 20NE correctly identified 87.0% of 56<sup>63</sup> and 99.0% of 800 test isolates<sup>61</sup> respectively in two studies. It should be considered that the isolates tested in these studies were probably originally identified as *B. pseudomallei* by biochemical means. Another study comparing API 20NE results with molecular testing found that only 37% of API 20NE results correctly identified *B. pseudomallei* after 48 h incubation<sup>54</sup>.

Matrix-assisted laser desorption/ionization of time-of-flight mass spectrometry (MALDI-TOF MS) has shown potential for rapid detection of *B. pseudomallei* at a low cost<sup>64</sup>. In order to identify *B. pseudomallei* using the MALDI-TOF system, a MALDI Biotyper Security Reference Library must be installed on the analyser. A recent paper by Inglis et al. described both the development of an in-house *B. pseudomallei*

mass spectra pattern library employing 43 isolates identified by *lpxO* PCR assay and gas-liquid chromatography bacterial fatty acid methyl ester analysis. Employment of this library in clinical practice led to the detection of *B. pseudomallei* directly from the positive blood cultures of two patients<sup>64</sup>.

Non-commercial monoclonal antibody solutions have been successfully employed in initial *B. pseudomallei* screening. When compared to a semi-nested PCR as a reference method, monoclonal antibody testing after 24 h growth yielded positive results for 87% of 71 PCR positive isolates. This rose to 94% of PCR positive isolates being correctly identified using monoclonal antibodies when 48 h colonies were tested<sup>54</sup>. It should be noted that cross-reaction of the monoclonal antibody reagent with *Burkholderia multivorans* and *Burkholderia thailandensis* was observed in this study<sup>54</sup>.

Cellular fatty acid analysis using 2-hydroxymyristic acid as a marker for *B. pseudomallei* has shown moderate success<sup>54</sup>, although the need for specialised gas-liquid chromatographs, cross-reactions with *B. cepacia*, 3 day delay for results and the need to repeat tests on some occasions render this method impractical for most laboratories.



Two species that may act as confounders to the identification of *B. pseudomallei* in the laboratory are *Burkholderia oklahomensis* and *Burkholderia thailandensis*<sup>65,66</sup>. Both represent environmental organisms almost identical biochemically and morphologically to *B. pseudomallei*, but not significantly associated with human disease, and rarely are they encountered in clinical specimens<sup>62</sup>. *B. thailandensis* may be differentiated from *B. pseudomallei* by its capacity to assimilate arabinose<sup>67</sup>. However, *B. oklahomensis* requires molecular approaches to be differentiated from *B. pseudomallei*<sup>62</sup>.

Highly resistant small-colony variants of *B. pseudomallei* have previously been induced in vitro, but have not yet been detected in any clinical infections<sup>68</sup>. However, a recent concerning development was the isolation of ceftazidime resistant variants of *B. pseudomallei* with abnormal growth defects that negate their growth on routine non-selective agars. Such variant strains were isolated alongside wild-type *B. pseudomallei* from several patients unresponsive to ceftazidime therapy<sup>69</sup>. These variants do not grow on blood or MacConkey agars and grow only slowly on Ashdown's medium (it is thought that the buffering effect of glycerol in Ashdown's medium allows growth). Colonies are pinpoint size after 48 h growth on Ashdown's agar, and do not grow sufficiently quickly for biochemical identification; therefore, a molecular approach is required<sup>69</sup>.

### ***Molecular detection and identification***

A number of PCRs have been described in the literature<sup>70-72</sup>. Merritt et al. compared three novel PCR assays (once conventional and two real-time assays) using a previously published semi-nested conventional real-time PCR as the reference method<sup>70</sup>. A Taqman PCR directed against *lpxO* was identified as the most sensitive and specific assay<sup>70</sup>.

Two Taqman real-time PCR assays developed in Thailand (designated 8653 and 9438) showed 100% specificity and sensitivity when tested against bacterial extracts and crude lysates. When these two PCRs were used to detect *B. pseudomallei* in clinical sputum and blood specimens, PCR 8653 showed superior sensitivity, particularly in fatal cases<sup>71</sup>.

The findings of the study highlight the need to determine sensitivity and specificity of PCR detection in clinical isolates and not bacterial lysates or spiked samples.

### ***Serology***

The most sensitive serological test for antibodies to *B. pseudomallei* available at present is the indirect haemagglutination assay<sup>73</sup>. However, sensitivity is adversely affected by high background prevalence of positive antibodies in endemic areas, and thus the diagnostic cut-off varies based on local prevalence of melioidosis. In Australia, this is set at a level of  $\geq 1:40$  (with a 56% sensitivity), compared against 1:160 in north-eastern Thailand (with a 72% sensitivity)<sup>74</sup>. In Thailand, a specific direct immunofluorescent antibody test (IFAT) and latex agglutination is available but this is not commonly used in Australia<sup>75-78</sup>. The latex agglutination test performed well in a small sample volume study ( $n=30$  isolates) comparing the test to PCR, with 100% correlation in results found<sup>55</sup>. In a separate and much larger study ( $n=800$  isolates), 99.5% of *B. pseudomallei* isolates reacted in the latex agglutination test. The test was negative for 120 other oxidase positive, Gram negative bacilli. The test did not differentiate between *B. mallei* and *B. pseudomallei*, and the methods used by contributing laboratories to identify submitted *B. pseudomallei* were not stated<sup>61</sup>. A whole-cell antigen IFAT derived from *B. thailandensis* performed well when compared to *B. pseudomallei* whole-cell antigen IFAT, with advantages for assay preparation due to the low pathogenicity of *B. thailandensis*<sup>76</sup>. This highlights the possibility that prior patient exposure to soil organisms similar to *B. pseudomallei*, such as *B. thailandensis*, may result in false positive serosurvey results. The need to apply caution in the interpretation of serological results when employing unvalidated ELISA methods was recently highlighted by Peacock et al<sup>73</sup>.

### ***Controversies in laboratory identification***

The ideal method of *B. pseudomallei* identification is unclear. Whilst some studies show a high accuracy of biochemical tests combined with simple screening tests<sup>61,79,80</sup>, this has been called into question by other studies<sup>54,63,80</sup>. There is significant





interstudy variation in results, and some authors now suggest that biochemical testing panels have a limited role in *B. pseudomallei* identification due to the low sensitivity of this methodology<sup>54</sup>.

There is evidence showing that monoclonal antibody-based latex agglutination tests have excellent sensitivity and specificity<sup>55,61,81-83</sup>, with the exemption of urine antigen testing, which has been shown to have 100% specificity but low sensitivity<sup>75</sup>. Only one study reported a lower sensitivity compared to PCR identification methods<sup>54</sup>. This tends to be more cost-effective and is routinely used in endemic areas. However in non-endemic regions where laboratory technicians lack experience in identifying *B. pseudomallei* and latex agglutination tests will not be routinely available in diagnostic labs, positive identification of a suspected isolate can be difficult.

A 'gold standard' identification method has not yet been determined; it would assist greatly in addressing the above controversies<sup>84</sup>. Molecular testing has shown excellent sensitivity and specificity, and is gaining wider acceptance due to its rapid rate of detection<sup>84,85</sup>. Furthermore, differentiating between *B. pseudomallei* and closely related species such as *B. mallei* and *B. thailandensis* has been shown to be an issue in the past with other diagnostic techniques – molecular techniques allow us to address this issue<sup>86,87</sup>. They can also reduce exposure of lab personnel to the pathogen when compared to more conventional diagnostic methods<sup>82</sup>. However, molecular methods are not always readily available, particularly in developing countries. Also, random mutation of the primer binding site may lead to unreliable results with molecular techniques. A recently employed method of identification is the use of MALDI-TOF MS; however, it is crucial to expand the existing databases with pathogens endemic to different regions, because the current commercial databases may result in rare instances of species misidentification<sup>88</sup>.

## Clinical treatment

Clinical management has two main phases: the intravenous intensive phase for treatment of acute disease, followed by

the eradication phase. At the moment, intravenous ceftazidime (2 g, 6 hourly) or meropenem (1 g, 8 hourly) plus high-dose cotrimoxazole are the drugs of choice in Australia, and it is usually administered for at least 14 days<sup>89,90</sup>. One of the problems with melioidosis is that it is intrinsically resistant to many broad-spectrum antibiotics (such as penicillin, ampicillin, gentamicin, streptomycin, and first- and second-generation cephalosporins). Most strains are, however, sensitive to newer beta-lactams, ceftazidime, imipenem, meropenem, piperacillin, amoxicillin–clavulanate, ceftriaxone and cefotaxime. Prior to 1989, conventional therapy was often a combination of various drugs (chloramphenicol, cotrimoxazole, doxycycline, trimethoprim, sulfamethoxazole) given for 6 weeks to 6 months. Ceftazidime was associated with a significantly lower rate of mortality in severe melioidosis<sup>91</sup>. In a comparison trial, the overall therapeutic failure rate was significantly higher for those treated with amoxicillin–clavulanate<sup>92</sup>. It was concluded that whilst amoxicillin/clavulanate was a safe and effective initial treatment, ceftazidime was the treatment of choice for severe melioidosis<sup>92</sup>. The addition of cotrimoxazole did not confer a short nor long-term benefit<sup>93,94</sup>. Cefoperazone/sulbactam has been compared against ceftazidime as adjunctive therapy to cotrimoxazole, and there was no significant difference between mortality rate, duration of defervescence and bacteriological response in the two treatment arms<sup>95</sup>. Antibiotics other than ceftazidime may be used as a second-line therapy where first-line treatment options are unavailable or contraindicated<sup>96,97</sup>. Resistance to ceftazidime, amoxicillin–clavulanate and carbapenems in clinical cases is rare, and issues with resistance are more pertinent in eradication therapy<sup>90,98,99</sup>. In Australia, carbapenems (imipenem and meropenem) are alternatives to ceftazidime as first-line therapy. This was initiated after they were shown to be highly active in vitro<sup>100-102</sup>. It has been shown that there was no difference in overall or short-term survival and that treatment failure may be more common in those with ceftazidime alone<sup>103</sup>. Further research is required to investigate if meropenem is indeed of higher efficacy than ceftazidime, and the ATOM (Acute Treatment of Melioidosis) trial is currently under way to address this issue



(US National Institutes of Health ClinicalTrials Identifier NCT00579956).

## Eradication therapy

Three months of oral antibiotic therapy is usually the minimum for the eradication phase, and this is further lengthened in severe infections. In the past, a four-drug regimen (trimethoprim, sulfamethoxazole, doxycycline, chloramphenicol) was employed. This regimen was not well tolerated, which led to low adherence to therapy. It has been shown that in patients who survive the initial disease, choice and duration of antibiotic therapy is the most important determinant of relapse, with up to 90% decreased risk of relapse in those who adhere to an appropriate regimen for 12–16 weeks versus 8 weeks<sup>104</sup>. In Australia, the drug of choice is oral monotherapy with high-dose cotrimoxazole (320 mg of trimethoprim and 1600 mg of sulphamethoxazole, 12 hourly), whereas in Thailand a weight-based dosing protocol has now come into effect (<40 kg body weight, 160/800 mg; 40–60 kg, 240/1200 mg; >60 kg, 320/1600 mg – all 12 hourly), in combination with doxycycline (100 mg, 12 hourly)<sup>90,91,105</sup>. If this cannot be tolerated, or is contraindicated, amoxicillin–clavulanate (500 and 125 mg respectively, 8 hourly) is employed, using a weight-based dosing protocol (20 mg and 5 mg respectively/kg, thrice daily)<sup>96,106,107</sup>. Resistance to cotrimoxazole in *B. pseudomallei* does occur, although this is quite rare in Australia (2.5% of clinical isolates)<sup>96</sup> in comparison with other areas (13–16% in Thailand)<sup>108,109</sup>. Resistance over the course of therapy has also been documented, and this can be quite difficult to manage because there is often cross-resistance to other agents<sup>109</sup>.

Post-exposure prophylaxis may be considered in some cases, particularly in accidental laboratory exposure. However, it must be noted that evidence for efficacy in humans is lacking. In animal models it has been shown that animals receiving cotrimoxazole had a 100% survival rate if it was given within 24 h. Currently it is thought that cotrimoxazole (320 mg of trimethoprim and 1600 mg of sulphamethoxazole, 12 hourly

for 3 weeks)<sup>90</sup> would be the best first-line agent, with doxycycline and amoxicillin–clavulanate as alternatives.

## Conclusions

Our understanding of melioidosis has come a long way since the discovery of the disease nearly 100 years ago by Whitmore. However, it continues to be a lethal disease causing considerable mortality and morbidity in hyper-endemic areas. Increasing prevalence combined with the fact that a highly effective treatment has not yet been found makes it an important public health entity in these countries. Continued education and clinical awareness by medical professionals operating in endemic countries or working with those returning from endemic areas remains the most effective means by which to ensure timely identification and appropriate management of cases of melioidosis.

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