

Melioidosis: An emerging infectious disease

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ABSTRACT

Infectious diseases account for a third of all the deaths in the developing world. Achievements in understanding the basic microbiology, pathogenesis, host defenses and expanded epidemiology of infectious diseases have resulted in better management and reduced mortality. However, an emerging infectious disease, melioidosis, is becoming endemic in the tropical regions of the world and is spreading to non-endemic areas. This article highlights the current understanding of melioidosis including advances in diagnosis, treatment and prevention. Better understanding of melioidosis is essential, as it is life-threatening and if untreated, patients can succumb to it. Our sources include a literature review, information from international consensus meetings on melioidosis and ongoing discussions within the medical and scientific community.

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The name melioidosis [also known as Whitmore disease] is taken from the Greek word 'melis' meaning distemper of asses and 'eidos' meaning resembles glanders. Melioidosis is a zoonotic disease caused by *Pseudomonas pseudomallei* [now known as *Burkholderia pseudomallei*], a bacillus that can cause disease in horses, goats, pigs, lambs, cows, and other animals, as well as in humans.^[1,2] There have been concerns of *B. pseudomallei* being thought of as a potential biological warfare agent.^[3]

Prevalence and epidemiology

B. pseudomallei, the causative organism of melioidosis, is a free-living gram-negative, aerobic bacillus that is mainly widespread in Southeast Asia [most cases in Thailand, Malaysia, Vietnam, Cambodia, Laos, and Myanmar] and Northern Australia.^[4,5] There are some reports of its presence in other parts of the world such as Central America, the Caribbean, China, Taiwan, Africa, the Middle East and South Asian countries.^[6,7,8,9,10] In Thailand 2000 to 3000 new cases are diagnosed every year.^[11] In Malaysia, reported seroprevalence in healthy individuals is 17-22% in farmers [mainly rice farmers] and 26% in blood donors.^[12] In North Australia 0.6 to 16% of children have evidence of infection by *B. pseudomallei*.^[13] The *B. pseudomallei* have also been isolated in America.^[3] There are reports of several cases of patients with melioidosis who have immigrated into Europe and the disease has been increasingly recognized

in returning travellers to Europe from endemic areas.^[14] The geographic area of the prevalence of the organism is bound to increase as the awareness increases.

B. pseudomallei is a natural inhabitant of soil and water in the tropics and subtropics but can also survive in dry atmospheric conditions. It is ubiquitous in the rice-farming areas. It is also present in rubber plantations, cleared fields, cultivated and irrigated agricultural sites as well as drains and ditches. Its true prevalence however is not known, as there is under-reporting of its incidence due to the poorly understood disease process and misdiagnosis. At the same time enough resources are not always available in some areas to carry out research and increase the awareness of the general public and to educate and familiarise the medical profession about the disease.

Melioidosis affects all ages but peak incidence is mainly between 40 to 60 years of age, with male to female ratio of 1.4:1.^[15] There is a good correlation between the isolation of the organism from soil and the seroprevalence of antibodies in the population living in that region.

Bacteriology

When isolated from blood, sputum, pus and other body fluids, *B. pseudomallei* appears like safety pins [bipolar] under the microscope with methylene blue stain. It grows aerobi-

cally on ordinary media at 37°C. Colonies are wrinkled and show dry daisy-head appearance along with a distinct odour. Mucoid colonies suggest that the patient is receiving antibiotic therapy. In laboratory culture of *B. pseudomallei*, growth of other organisms may result in false negative result. This problem could be resolved using Ashdown's selective medium, which contains dyes, gentamicin and Trypticase peptone.^[16] Recently, more improved *B. pseudomallei* selective agars [BPSA] have been developed to improve the recovery of *B. pseudomallei*.^[17]

B. pseudomallei can survive anaerobic conditions in the presence of acidic environment, and also survive in distilled water for several years.^[18] The bacterium is resistant to penicillin, aminoglycosides, rifamycins and relatively insensitive to quinolones and macrolides. Therefore the therapeutic options are limited and continuous presence of the organism in patients is not fully understood.^[19,20] *B. pseudomallei* is resistant to macrolide and aminoglycoside antibiotics via a multidrug efflux pump.²⁰ Mutations within the conserved motifs of the beta-lactamase enzyme [enzyme that hydrolyses the cyclic amide bond of beta-lactam antibiotics] also account for the resistance patterns.^[21]

Modes of transmission and pathogenesis

There are several established modes of transmission within the patient population. The possible modes are inhalation, ingestion or inoculation through the skin lesions from the contaminated soil.^[22] Person-to-person transmission of *B. pseudomallei* especially between patient and his sibling or one of their playmates is common.^[23] Vertical transmission [from mother to child] is possible.^[24] It can also be transmitted by direct contact with infected rodents or infected food, soil, water, excreta; person-to-person transmission is also possible through use of injection needle. *B. pseudomallei* can also be transmitted through sexual intercourse.^[25] The link between melioidosis and consumption of Kava [*Piper methysticum*] has also been seen.^[26] Heavy rains and winds may cause increased inhalation of *B. pseudomallei*.^[27] Interestingly, a container of commercial hand-wash detergent was a source of infection in Northern Australia.^[28]

B. pseudomallei attack several eukaryotic cell lines. In both phagocytic and nonphagocytic cell lines, it can escape from the specialised endocytic vacuoles into the cytoplasm to form actin-associated membrane protrusion that is thought to contribute to cell-to-cell spreading in the infected individuals.^[29,30,31] Capsule and a type III secretion system [TTSS-expressed mainly by pathogenic bacteria that is used to introduce deleterious proteins called effectors into host cells] facilitate *B. pseudomallei* to survive, escape from endocytic vesicles, facilitate bacterial invasion of epithelial cells and intracellular survival.^[32,33] The uptake of *B. pseudomallei* by several cell lines in culture leads to induction of cell fusion and formation of a multinucleated large cell.^[34] Production of nitric oxide has bactericidal activity and failure of infected cells to successfully control the growth and subsequent survival of in-

tracellular *B. pseudomallei* are due to the suppression of inducible nitric oxide synthase [iNOS] by *B. pseudomallei*.^[35,36] However, interferons enhance antimicrobial activity of macrophage infected *B. pseudomallei* by up-regulating iNOS.^[36,37]

Clinical manifestations

B. pseudomallei can cause disease in apparently healthy individuals. Clinical manifestations of melioidosis range from localised infection to acute pneumonia and fulminant septic melioidosis.^[38,39,40] Once infected, it may remain dormant and become active after months, years or decades when host is immunocompromised by drugs [steroids] or disease [diabetes mellitus, chronic renal failure, retrovirus infections, haematological malignancies, collagen vascular disease] or social deprivation [alcoholism, drug abuse, occupational exposure].^[41] The factors that provoke the reactivation of latent pathogen probably are environmental variables, stress and immunity status.^[42,43] Localized melioidosis occurs in the form of acute suppurative lesions, superficial and deep-seated abscess in the psoas muscle, parotid glands and at the root of mesentery.^[44,45] It may also present as cellulitis, chronic otitis media and sepsis after burns and trauma.^[45,46] The other manifestations are mycotic aneurysm, pericarditis, osteomyelitis epididymo-orchitis and prostatitis.^[47,48,49] Melioidosis is also associated with systemic lupus erythematosus.^[50] Melioidotic prostatic abscesses are reported very rarely and are not easy to diagnose. In endemic areas, the elderly diabetic person who presents with high-grade fever and urinary obstruction may have *B. pseudomallei* in the prostate gland.^[51] Central nervous system involvement including brain abscess is a rare complication with high mortality.^[52,53] The immune-suppressed patients present with melioidosis septicaemia and their clinical features are similar to other gram-negative septicaemias and its prognosis is poor. Quoted mortality ranges from 40% to 75% despite rational use of anti-microbial therapy.^[54] Clinical risk factors and manifestations of melioidosis are summarized in Table 1.

Diagnosis

The diagnosis of acute or chronic melioidosis remains challenging. In endemic areas, melioidosis should be considered in the differential diagnosis of any Pyrexia of Unknown Origin [PUO], acute respiratory distress syndrome [ARDS] and acute septicaemia. The other conditions that melioidosis may present as are pneumonia, acute suppurative lesions, chronic granulomatous lesions, septic arthritis, osteomyelitis, epididymorchitis and mycotic aneurysm as well as radiological pattern of tuberculosis on the chest X-ray but not supplemented with mycobacterium tuberculosis positive sputum culture. In diabetic patients, laboratory results show leukocytosis, high level of glucose in blood and glycosylated haemoglobin, and high urea and creatinine.^[55] In melioidosis, laboratory diagnosis is essential for successful patient management. C-reactive protein [CRP], an early indicator of infectious or inflammatory conditions may be elevated in melioidosis; however under normal CRP levels, melioidosis should not be ruled out.^[56]

Table 1: Clinical risk factors for infection and clinical manifestation of melioidosis

Clinical risk factors	Diabetes mellitus, excessive alcohol consumption, chronic renal impairment, cystic fibrosis, chronic heart failure, chronic pulmonary disease, leukaemia and lymphoma, corticosteroid therapy, immunodeficiency diseases, neoplasms, kava consumption
Affected Organ Systems and Clinical manifestations	
<i>Cardiovascular:</i>	Pericarditis, pericardial effusion, endocarditis, endarteritis
<i>CNS:</i>	Meningitis [primary], encephalitis, intracranial abscess
<i>Genitourinary:</i>	Urinary tract infection [pyelonephritis], prostatitis or prostatic abscess, epididymorchitis, perinephric abscess, scrotal abscess
<i>Lymphatic:</i>	Lymphadenitis or abscess
<i>Hepatobiliary:</i>	Liver abscess, splenic abscess, cholangitis, pancreatic abscess
<i>Respiratory:</i>	Pneumonitis, lung abscess, pleural effusion, empyema, miliary granuloma
<i>Skeletal:</i>	Septic arthritis [knee, elbow, ankle joints], osteomyelitis, Subperiosteal abscess
<i>Skin and soft tissue:</i>	Cellulitis, subcutaneous abscess, infected wound, chronic granuloma, ecthyma gangrenosum, hemorrhagic bleb, chronic pustules, pyomyositis, urticaria, mastitis
<i>Others:</i>	Prolonged pyrexia without obvious source, septicemia, ophthalmitis, parotid abscess, corneal ulcers

Identification of *B. pseudomallei*

Isolation of *B. pseudomallei* by culture from a clinical specimen [blood, urine, sputum, skin lesions and swab samples from throat] is the gold standard of diagnosis.^[57] Correct identification of *B. pseudomallei* is essential for long term supportive therapy in the treatment of melioidosis. A few simple tests can be employed to identify *B. pseudomallei* in the endemic areas. These tests include positive oxidase test, bipolar gram staining, metallic sheen colonies on special media (Ashdown media which contains various dyes and gentamicin) and resistance to aminoglycosides.^[58]

Conventional biochemical tests and API20E substrate-utilization test panel [bioMérieux] kit is used for identification of *B. pseudomallei*; however, it can easily misidentify *Chromobacterium violaceum* (*C. violaceum*). In one study, polymerase chain reaction (PCR) results showed that isolated *C. violaceum* have similar repetitive extragenic pallindromic sequence (REPS) pattern with *B. pseudomallei*.^[59]

Serological tests

Serological tests are helpful in making a provisional diagnosis in the absence of isolation of *B. pseudomallei* in the specimen. Culture and serological methods are cost-effective and simple to perform but require experience to interpret results. Slide

agglutination test results in rapid identification of *B. pseudomallei*. Indirect haemagglutination test is simple to perform as it detects the antibody against *B. pseudomallei* that appears in the blood within 1-2 weeks after the infection and reach maximal titre in 4 to 5 months.^[60] However its interpretation may be difficult because of the following points;

- False positive results due to cross-reaction with other gram negative bacteria [shares antigens (lipopolysaccharide of cell wall) particularly *Burkholderia cepacia* and *Legionella species*],
- There may be rare false negative results,
- High antibody titre may persist for a long time after infection subsides.

Enzyme linked immunosorbent assay [ELISA] test detects specific IgG and IgM antibodies of *B. pseudomallei* in serum specimens. ELISA is more convincing in terms of sensitivity and specificity for antibody detection as it points to an active disease process.^[61] The indirect ELISA is easy to perform and hence is recommended as a diagnostic serological test when melioidosis is in the differential diagnosis of PUO cases. Though Immunofluorescent Antibody Assay is a rapid, highly sensitive and specific test for the identification of current infection, it requires a fluorescent microscope that is not always available in some laboratories in endemic areas.

Molecular identification techniques

During the past decade, many efforts have been made to develop new molecular procedures to identify *B. pseudomallei* from various specimens. Molecular biology techniques such as polymerase chain reaction [PCR], dot immunoassay, pulsed field gel electrophoresis [PFGE], restricted fragmentation length polymorphism [RFLP] and random amplification of particle of deoxyribonuclease [RAPD] are also used for diagnosis. These are the recommended techniques for the rapid diagnosis of the disease and for monitoring therapy and epidemiological studies because of its high sensitivity, specificity, simplicity and speed. In recent times sensitive PCR amplification techniques for detecting the DNA of *B. pseudomallei* in clinical specimens, especially buffy coat specimens of acute melioidosis patients have been useful.^[62,63] Laboratory diagnostic approach is summarized in Table 2.

Management

The main objective of treatment is to reduce the mortality and morbidity in melioidosis. Before the advent of proper antimicrobials, the mortality of the melioidosis patients used to be around 95%. Rational use of antimicrobials has reduced it to half. Ceftazidime is the drug of choice in systemic melioidosis.^[64,65] Ceftazidime [120mg/kg/day], has shown to reduce the mortality significantly in severe melioidosis.^[66] However, resistant strains are beginning to appear.¹⁹ Carbapenem antibiotics are also suitable for the treatment of the disease.^[67] Doxycycline can be used in localised infections in combination with cotrimoxazole.^[68] In acute severe melioidosis, ceftazidime alone or in combination with co-trimoxazole or ciprofloxacin remains the drug of choice. Parenteral amoxycylav

Table 2. Laboratory diagnosis of melioidosis

Clinical laboratory results	Isolation of <i>B. pseudomallei</i>	Identification of <i>B. pseudomallei</i>	Serological tests	Molecular methods to detect <i>B. pseudomallei</i> DNA
Increased C-reactive protein, leukocytosis. In diabetic patients, high level of glucose in blood, high urea and creatinine and glycosylated haemoglobin.	Growth of metallic sheen colonies on Ashdown media from clinical specimens	Positive oxidase test, bipolar staining of gram negative rods, resistance to aminoglycosides. Conventional biochemical tests. API 20NE, API 20 E.	Enzyme linked immunosorbent assay [ELISA] to detect IgM & IgG antibodies. Indirect haemagglutination test to detect antibodies against <i>B. pseudomallei</i> . Immunofluorescent Antibody Assay.	PCR, dot immunoassay, PFGE, RFLP, RAPD

[160mg/kg/day for 8 weeks] is a substitute of ceftazidime and it reduces mortality.^[69] Imipenem is a safe and effective treatment for acute severe melioidosis and may also be considered an alternative to ceftazidime.^[70,71] A study showed that meropenem [1g or 25 mg/kg, 8 hourly intravenously for ≥ 14 days] can be considered as an alternative to ceftazidime and imipenem in the treatment of melioidosis but this is more expensive and more trials are required.^[72]

In melioidosis, supportive therapy is an integral part of management for better prognosis as it includes management of shock and ARDS, drainage of pus, good control of diabetes in diabetics and good nursing care. The recommendation for parenteral antimicrobial therapy, when needed, is for at least two weeks before switching over to oral maintenance therapy for 12 to 20 weeks. [Table 3]

Relapse and maintenance therapy

Appropriate treatment is imperative in order to prevent relapse and failure of treatment. Despite appropriate treatment, melioidosis has a higher relapse rate. The average time between discharge from hospital and relapse is of 21 weeks. Treated patients require long-term follow up, as *B. pseudomallei* re-

Table 3: Antimicrobial therapy for treating severe melioidosis ^[55, 74,75]

Severe melioidosis
Ceftazidime 100-120 mg/kg/day in 3 divided doses + Co-trimoxazole 8-12 and 40-60 mg/kg/day
Or
Ceftazidime + Ciprofloxacin 500mg 12 hourly (only 2 weeks)
Bacteremia only
Ceftazidime monotherapy
Or
Ceftazidime + Co-trimoxazole or doxycycline 4mg/kg/day
Localised melioidosis
Amoxicillin clavulanate 160mg/kg/day
Or
Co-trimoxazole + Doxycycline
Oral maintenance therapy
Co-trimoxazole, doxycycline with or without the initial 4 weeks of chloramphenicol
Or
Amoxicillin clavulanate

mains latent for up to 26 years in the body.^[73] For maintenance therapy, Co-Amoxycylav is a safe and well-tolerated antimicrobial agent [there is some concern that it may be less effective than the conventional regimen of chloramphenicol, cotrimoxazole and doxycycline]. The recommended duration for maintenance therapy is of 12 to 20 weeks.^[74,75]

It has been shown that *B. pseudomallei* stays intracellularly in the body where it produces biofilms and micro colonies and is sheltered from β -Lactam antimicrobial drugs [β -Lactam drugs are unable to enter intracellular sites to kill latent *B. pseudomallei*].^[76] It has been suggested that a combination of ciprofloxacin and macrolides is a good alternative regimen since ciprofloxacin penetrates phagocytic cells and achieves intracellular concentrations of several times higher than extracellular concentration and kills *B. pseudomallei* while macrolides could delay or prevent production of glycocalyx [Table 3].^[77,78]

Prevention

Measures for prevention require prompt cleansing of scrapes, burns, or other open wounds in endemic areas. Persons with diabetes and skin lesions should avoid contact with soil and standing-water in endemic areas. Protective clothing such as rubber boots and gloves during agricultural work can prevent infection through the feet and hands. It is important to maintain safe water through regular disinfection and safe storage of water for both human and animals bred for human consumption. Sewage wastes can attract insects and rodents and encourage the growth of *B. pseudomallei*. Therefore proper disposal of sewage wastes is essential in endemic areas. As dairy products can contain *B. pseudomallei* it is important that milk is pasteurized before consumption.^[79]

There is currently no licensed vaccine available for protection against melioidosis. At present studies are underway to identify possible antigens using lipopolysaccharides of *B. pseudomallei* in mouse models.^[80] Antibodies against *B. pseudomallei* flagellin reduce the motility of the bacterium and provide protection against melioidosis in animal models.^[81] A recent study has shown that quicklime was able to inhibit the growth of *B. pseudomallei* in soil from a rice field.^[82] As our understanding of the disease increases and as we move forward with the studies on the pathogenesis of the disease, new and effective vaccine against melioidosis may become a reality.

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