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Revised 03/15/2015

MELIOIDOSIS MANUAL

Melioidosis, also called Whitmore's disease, is an infectious disease that can infect humans or animals. It is also capable of infecting plants. The disease is caused by the bacterium *Burkholderia pseudomallei*.

Microbiology

B. pseudomallei is a saprophytic, bipolar, aerobic, motile rod-shaped bacterium widely distributed in tropical soil and water. It was previously classed as part of the *Pseudomonas* genus and until 1992, it was known as *Pseudomonas pseudomallei*. It is phylogenetically related closely to *B. mallei,* which causes glanders, an infection primarily of horses, donkeys, and mules. The name melioidosis is derived from the Greek melis (μηλις) meaning "a distemper of asses" with the suffixes -oid meaning "similar to" and -osis meaning "a condition", that is, a condition similar to glanders

B. pseudomallei measures 2 μm -5 μm in length and 0.4 μm -0.8 μm in diameter and is capable of self-propulsion using flagellae. The bacteria can grow in a number of artificial nutrient environments, especially betaine- and arginine-containing ones. In vitro, optimal proliferation temperature is reported around 40°C in neutral or slightly acidic environments (pH 6.8 - pH 7.0).

B. pseudomallei is not fastidious and will grow on a large variety of culture media (blood agar, MacConkey agar, EMB, etc.). Ashdown's medium (or *B. cepacia* medium) may be used for selective isolation. Cultures typically become positive in 24 to 48 hours. (This rapid growth rate differentiates the organism from *B. mallei*, which typically takes a minimum of 72 hours to grow). Colonies are wrinkled, have a metallic appearance, and possess an earthy odor. On Gram staining, the organism is a Gram-negative rod with a characteristic "safety pin" appearance (bipolar staining). On sensitivity testing, the organism appears highly resistant (innately resistant to a large number of antibiotics including colistin and gentamicin), and that again differentiates it from *B. mallei*, which is in contrast, exquisitely sensitive to a large number of antibiotics.

Laboratory identification of *B. pseudomallei* can be difficult, especially in Western countries where it is rarely seen. The large wrinkled colonies look like environmental contaminants, so are often discarded as being of no clinical significance. The pattern of resistance to antimicrobials is distinctive, and also helps to differentiate the organism from *P. aeruginosa*. The majority of *B. pseudomallei* isolates are **intrinsically resistant to all aminoglycosides** (via an efflux pump mechanism, but sensitive to co-amoxiclav; this pattern of resistance almost never occurs in *P. aeruginosa* and is helpful in identification.

History

Alfred Whitmore and assistant Krishnaswami first reported the disease among beggars and morphine addicts at autopsy in Rangoon, present-day Myanmar, in a report published in 1912. They distinguished it from glanders, similar in presentation, but caused by a different microorganism. *B. pseudomallei*, the

Whitmore bacillus, was identified in 1917 in Kuala Lumpur. Sir Arthur Conan Doyle may have read the 1912 report before writing a short story that involved the fictitious tropical disease "tapanuli fever" in a Sherlock Holmes adventure.

Other names for melioidosis are Pseudoglanders, Whitmore's disease (after Captain Alfred Whitmore, who first described the disease), Nightcliff gardener's disease (Nightcliff is a suburb of Darwin, Australia where melioidosis is endemic), paddy-field disease, and morphia injector's septicaemia.

The history of melioidosis in Thailand demonstrates the degree to which a highly endemic infection may go unrecognized in the absence of clinical awareness and appropriate laboratory facilities. The earliest evidence for the existence of the melioidosis in Thailand (then Siam) was provided by Gambier, who isolated *P. pseudomallei* from a Russian patient normally domiciled in Bangkok. In 1947, two further cases were reported from Siam, in a Dutch prisoner of war and an Indian soldier, but it was not until 1955 that the first case was reported in a Thai. In 1962, a serological survey showed that up to 29.1% of healthy Thai volunteers had evidence of exposure to *P. pseudomallei*, but by 1966 only three cases of clinical melioidosis had been reported. By 1986 the Thailand Infectious Disease Association, had collected over 800 case reports. The disease in Thailand predominantly affects rice farmers and their families, who are thought to contract infection through their daily contact with the soil and water of paddy fields, in which *P.pseudomallei* exists as a saprophyte.

Reservoir

The bacteria is found in soil and water in specific tropical areas. Since *B. pseudomallei* is normally found in soil and surface water, a history of contact with soil or surface water is, therefore, almost invariable in patients with melioidosis. The majority of patients who do have contact with infected soil suffer no ill effects. Even within an area, the distribution of *B. pseudomallei* within the soil can be extremely patchy, and competition with other Burkholderia species has been suggested as a possible reason. Contaminated ground water was implicated in one outbreak in northern Australia. Also implicated are severe weather events such as flooding, tsunamis and typhoons.

The disease is clearly associated with **increased rainfall**, with the number (and severity) of cases increasing following increased precipitation

Mode of Transmission

Transmission can occur through inhalation or subcutaneous inoculation, occasionally by ingestion; person-to-person transmission is rare via contact with the blood or body fluids of an infected person.

Communicability

Not communicable from person-to-person.

Incubation Period

The incubation period (time between exposure and appearance of clinical symptoms) is not clearly defined, but may range from one day to many years; generally symptoms appear **two to four weeks** after exposure. Sub-clinical infections are also possible. With a high inoculum, symptoms can develop in a few hours.

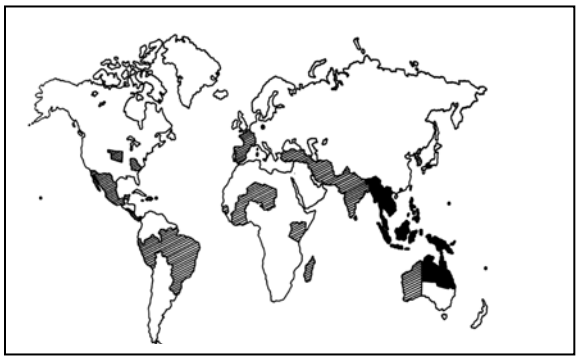
Epidemiologic Parameters

There is prevalence in endemic countries. Northeast Thailand has the highest incidence of melioidosis recorded in the world (21.3 cases of melioidosis per 100,000 people per year). In Northeast Thailand, 80% of children are positive for antibodies against *B. pseudomallei* by the age of four years; the figures are lower in other parts of the world.

Geographic Distribution

It is endemic in Southeast Asia, northern Australia, Papua New Guinea, much of the Indian subcontinent, southern China, Hong Kong, and Taiwan. It is considered highly endemic in northeast

Thailand, Malaysia, Singapore, and northern Australia. Melioidosis has been reported in Puerto Rico, suspected in El Salvador, and may be underdiagnosed in India, Africa, the Caribbean, and Central and South America. In northern Brazil, clusters of melioidosis have recently been recognized and are associated with periods of heavy rainfall.



While melioidosis infection has taken place all over the world, **Southeast Asia** (Thailand, Malaysia, Singapore) and **northern Australia** are the areas in which it is primarily found.

Though rarely reported, cases are thought to frequently occur in: Papua New Guinea, most of the Indian subcontinent, Southern China, Hong Kong, Taiwan, Vietnam, Indonesia, Cambodia, Laos, and Myanmar (Burma).

Outside of Southeast Asia and Australia, cases have been reported in: The South Pacific (New Caledonia), Sri Lanka, Mexico, El Salvador, Panama, Ecuador, Peru, Guyana, Puerto Rico, Martinique, Guadeloupe, Brazil, Parts of Africa and the Middle East.

In the **United States**, confirmed cases reported in previous years have ranged from zero to five and have occurred among travelers and immigrants coming from places where the disease is widespread. It has been found among troops of all nationalities that have served in areas with widespread disease.

L'affaire du Jardin des Plantes: The most extraordinary extension of the boundaries of melioidosis took place in France in the mid-1970s. Thanks to the chance visit of a researcher from the Institut Pasteur to a postmortem examination at the Museum Nationale d'Histoire Naturelle, an epizootic of melioidosis among animals in a Paris zoo was revealed. This outbreak subsequently spread to other zoos in Paris and equestrian clubs throughout France and beyond, probably by the transport of infected animals and contaminated manure. The infection caused the death or slaughter of an unknown number of animals and at least two fatal human cases. Extensive environmental contamination was documented, resulting in a costly program of disinfection despite which *P. pseudomallei* persisted for years in affected soil.

The origin of this outbreak is obscure. Possibly, infected horses from Iran imported the disease, or perhaps the index case was a panda donated to France by Mao-Tse-Tung in 1973. "L'affaire du Jardin des Plantes" is the only well-documented example of the transmission of melioidosis in a temperate climate and emphasizes the potential dangers of the importation of the disease to a new area. The isolation of *P. pseudomallei* from horses in Spain has also been reported, as have several possible cases of human melioidosis in Berlin in 1947, although the evidence for the last observation is uncertain.

Risk Factors

Behavioral risk factors

The risk is highest for military personnel, adventure travelers, ecotourists, construction and resource extraction workers, and other people whose contact with contaminated soil or water may expose them to the bacteria. Risk factors for systemic melioidosis include diabetes, excessive alcohol use, chronic renal disease, chronic lung disease (such as associated with cystic fibrosis or chronic obstructive pulmonary disease), thalassemia, and malignancy or other non-HIV-related immune suppression.

Medical risk factors

Although healthy people may get melioidosis, the major risk factors are:

- Diabetes
- Liver disease

- Renal disease
- Thalassemia
- Cancer or another immune-suppressing condition not related to HIV
- Chronic lung disease (such as cystic fibrosis, chronic obstructive pulmonary disease (COPD), and bronchiectasis)

Bioterrorism potential

Interest in melioidosis has been expressed because it has the potential to be developed as a biological weapon. It is classed by the US Centers for Disease Control and Prevention (CDC) as a category B agent. *B. pseudomallei*, like *B. mallei* which causes glanders, was studied by the US. as a potential biological warfare agent, but was never weaponized. The Soviet Union was reported to have also experimented with *B. pseudomallei* as a biological warfare agent.

Pathogenesis

B. pseudomallei is an "accidental pathogen". An environmental organism, it has no requirement to pass through an animal host to replicate. From the point of view of the bacterium, human infection is an evolutionary "dead end".

Strains which cause disease in humans differ from those causing disease in other animals by possessing certain genomic islands. It may have the ability to cause disease in humans because of DNA it has acquired from other microorganisms. The mutation rate is also high, and the organism continues to evolve, even after infecting the host.

B. pseudomallei is able to invade cells (it is an intracellular pathogen). It is able to polymerise actin and to spread from cell-to-cell, causing cell fusion and the formation of multinucleated giant cells. The bacterium also expresses a toxin called lethal factor 1. *B. pseudomallei* is one of the first Proteobacteria to be identified as containing an active type-6 secretion system. It is also the only organism identified that contains up to six different secretion systems.

B. pseudomallei is intrinsically resistant to a large number of antimicrobial agents. One important mechanism is that it is able to pump drugs out of the cell, and this mediates resistance to aminoglycosides (AmrAB-OprA), tetracyclines, fluoroquinolones, and macrolides (BpeAB-OprB).

Clinical Description

Clinical presentation of the disease varies on a case by case basis.

A patient with active melioidosis usually presents with **Fever**.
Pain or other symptoms may be suggestive of a **Clinical Focus**, found in around 75% of patients.

ACUTE MELIOIDOSIS

Fever & Pain: Lung, bone & cellulitis

➔cough or pleuritic chest pain suggestive of pneumonia,
➔bone or joint pain suggestive of osteomyelitis or septic arthritis,
➔localized pain cellulitis.

Fever, no pain in other presentations:

Localized abscesses:

- Intra-abdominal infection (including liver and/or splenic abscesses, or prostatic abscesses) do not usually present with focal pain, and imaging of these organs using ultrasound or CT should be performed routinely. In one series of 214 patients, 27.6% had abscesses in the liver or spleen (95% confidence interval, 22.0% to 33.9%). *B. pseudomallei* abscesses may have a characteristic "honeycomb" or "swiss cheese" architecture (hypoechoic, multiseptate, multiloculate) on CT.

- Regional variations in disease presentation are seen: parotid abscesses characteristically occur in Thai children, but this presentation has only been described once in Australia. Conversely, prostatic abscesses are found in up to 20% of Australian males, but are rarely described elsewhere. An encephalomyelitis syndrome is recognized in northern Australia.

- Melioidosis is said to be able to affect any organ in the body except the heart valves (endocarditis). Although meningitis has been described secondary to ruptured brain abscesses, primary meningitis has not been described. Less common manifestations include intravascular infection, lymph node abscesses (1.2% -2.2%), pyopericardium and myocarditis, mediastinal infection, and thyroid and scrotal abscesses and ocular infection.

Fever, no focal infection:

In up to 25% of patients, no focus of infection is found and the diagnosis is usually made on blood cultures or throat swab.

Patients with melioidosis usually have risk factors for disease, such as diabetes, thalassemia, hazardous alcohol use, or renal

CHRONIC MELIOIDOSIS

Chronic melioidosis is usually defined by a duration of symptoms greater than two months and occurs in about 10% of patients. The clinical presentation of chronic melioidosis is protean and includes such presentations as chronic skin infections, chronic lung nodule, and pneumonia. In particular, chronic melioidosis closely mimics tuberculosis, and has sometimes been called "**Vietnamese tuberculosis**".

Infection Classifications

Melioidosis can be categorized as:

- acute or localized infection,
- acute pulmonary infection,
- acute bloodstream infection,
- disseminated infection

Localized infection

This form generally presents as an ulcer, nodule, or skin abscess and may result from inoculation through a break in the skin and may produce fever and general muscle aches. The infection may remain localized, or may progress rapidly through the bloodstream.

Pulmonary infection

This is the most common form of presentation of the disease and can produce a clinical picture of mild bronchitis to severe pneumonia. Melioidosis may mimic tuberculosis, with fever, weight loss, productive cough, and upper lobe infiltrate, with or without cavitation. More than 50% of cases present with pneumonia. The onset of pulmonary melioidosis typical is marked by:

- high fever,
- headache,
- anorexia,
- general muscle soreness.
- Chest pain is common, but a nonproductive or productive cough with normal sputum is the hallmark of this form of melioidosis. Cavitary lesions may be seen on chest X-ray, similar to those seen in pulmonary tuberculosis.

Bloodstream infection

This is typically an infection with rapid onset, and abscesses may be found throughout the body, most notably in the liver, spleen, or prostate.

Patients with underlying risk factors such as diabetes and renal insufficiency are more likely to develop this form of the disease, which usually results in septic shock. The symptoms of bloodstream infection may include:

- fever,
- headache,
- respiratory distress,
- abdominal discomfort,
- joint pain,
- muscle tenderness,
- disorientation.

Disseminated infection

Disseminated melioidosis presents with abscess formation in various organs of the body, and may or may not be associated with sepsis. Organs involved typically include the liver, lung, spleen, and prostate; involvement of joints, bones, viscera, lymph nodes, skin, or brain may also occur. Disseminated infection may be seen in acute or chronic melioidosis. Signs and symptoms, in addition to fever, may include weight loss, stomach or chest pain, muscle or joint pain, and headache or seizure.

Laboratory tests / Diagnosis

Melioidosis is diagnosed by isolating *B. pseudomallei* from blood, urine, sputum, skin lesions, or abscesses; or by detecting an antibody response to the bacteria. *B. pseudomallei* is never part of the normal human flora.

Diagnostic assistance is available through the CDC (www.cdc.gov/ncezid/dhcpp/bacterial_special/zoonoses_lab.html).

A definite history of contact with soil may not be elicited, as melioidosis can be dormant for many years before manifesting. Attention should be paid to a history of travel to endemic areas in returned travelers. Some authors recommend considering possibility of melioidosis in every febrile patient with a history of traveling to and/or staying at endemic areas.

Culture

A complete screen (blood culture, sputum culture, urine culture, throat swab, and culture of any aspirated pus) should be performed on all patients with suspected melioidosis (culture on blood agar as well as Ashdown's medium). A definitive diagnosis is made by growing *B. pseudomallei* from any site. A throat swab is not sensitive, but is 100% specific if positive, and compares favorably with sputum culture.

The sensitivity of urine culture is increased if a centrifuged specimen is cultured, and any bacterial growth should be reported (not just growth above 104 organisms/ml which is the usual cutoff). Very occasionally, bone marrow culture may be positive in patients who have negative blood cultures for *B. pseudomallei*, but these are not usually recommended. A common error made by clinicians unfamiliar with melioidosis is to only send a specimen from the affected site (which is the usual procedure for most other infections) instead of sending a full screen.

Ashdown's medium, a selective medium containing gentamicin, may be required for cultures taken from nonsterile sites. *B. cepacia* medium may be a useful alternative selective medium in nonendemic areas, where Ashdown's is not available. A new medium derived from Ashdown, known as ‘Francis medium’, may help differentiate *B. pseudomallei* from *B. cepacia* and may help in the early diagnosis of melioidosis, but has not yet been extensively clinically validated.

Many commercial kits for identifying bacteria may misidentify *B. pseudomallei*.

Serology

Also, a serological test for melioidosis (indirect haemagglutination) is available, but not commercially in most countries. A high background titre may reduce the positive predictive value of serological tests in endemic countries. A specific direct immunofluorescent test and latex agglutination, based on monoclonal antibodies, are used widely in Thailand, but are not available elsewhere. Cross-reactivity with *B. thailandensis* is almost complete. A commercial ELISA kit for melioidosis appears to perform well but no ELISA test has yet been clinically validated as a diagnostic tool.

Imaging

It is not possible to make the diagnosis on imaging studies alone (X-rays and scans), but imaging is routinely performed to assess the full extent of disease. Imaging of the abdomen using CT scans or ultrasound is recommended routinely, as abscesses may not be clinically apparent and may coexist with disease elsewhere. Australian authorities suggest imaging of the prostate specifically due to the high incidence of prostatic abscesses in northern Australian patients. A chest X-ray is also considered routine, with other investigations as clinically indicated. The presence of honeycomb abscesses in the liver are considered characteristic, but are not diagnostic.

The differential diagnosis is extensive; melioidosis may mimic many other infections, including tuberculosis.

Treatment

Ceftazidime, imipenem, meropenem, trimethoprim-sulfamethoxazole, and doxycycline are commonly used. Relapse may be seen, especially in patients who choose a shorter than recommended course of therapy.

The type of infection and the course of treatment will impact long-term outcome. Treatment generally starts with intravenous (within a vein) antimicrobial therapy for 10 to14 days, followed by three to six months of oral antimicrobial therapy.

Antimicrobial agents that have been effective against melioidosis include:

- Intravenous therapy consists of:
    - Ceftazidime administered every 6-8 hours OR
    - Meropenem administered every 8 hours
- Oral antimicrobial therapy consists of:
    - Trimethoprim-sulfamethoxazole taken every 12 hours OR
    - Doxycycline taken every 12 hours

Patients with penicillin allergies should get an alternative treatment course.

Prognosis

Without access to appropriate antibiotics (principally ceftazidime or meropenem), the septicemic form of melioidosis exceeds 90% in mortality rate. With appropriate antibiotics, the mortality rate is about 10% for uncomplicated cases, but up to 80% for cases with bacteremia or severe sepsis. It seems certain that access to intensive care facilities is also important, and probably at least partially explains why total mortality is 20% in Northern Australia but 40% in Northeast Thailand. Response to appropriate antibiotic treatment is slow, with the average duration of fever following treatment being five to nine days.

Recurrence occurs in 10% to 20% of patients, but with co-trimoxazole eradication therapy, this can be reduced to 4%. While molecular studies have established the majority of recurrences are due to the original infecting strain, a significant proportion of recurrences (perhaps up to a quarter) in endemic areas may be due to reinfection, particularly after two years. Risk factors include severity of disease (patients with positive blood cultures or multifocal disease have a higher risk of relapse), choice of antibiotic for

eradication therapy (doxycycline monotherapy and fluoroquinolone therapy are not as effective), poor compliance with eradication therapy and duration of eradication therapy less than eight weeks.

Case Definition

Confirmed

A case that is laboratory confirmed, with or without clinical evidence.

- Isolation of *B. pseudomallei* from a clinical specimen of a case of severe febrile illness: culture of the organism may be done by blood, sputum, urine, pus, throat swab, or swabs from organ abscesses or wounds.
- Evidence of *B. pseudomallei* DNA (for example, by LRN-validated polymerase chain reaction) in a clinical specimens collected from a normally sterile site (blood) or lesion of other affected tissue (abscesses, wound).

Probable:

A case that meets the clinical case definition, one or more of the probable lab criteria, and one of the following epidemiologic findings:

- Epidemiologic finding for probable:
  - History of travel to a melioidosis-endemic region, OR
  - Known exposure to *B. pseudomallei* as a result of intentional release or occupational risk (lab exposure).
- Lab criteria for probable:
  Evidence of a fourfold or greater rise in *B. pseudomallei* antibody titer by IHA between acute- and convalescent-phase serum specimens obtained greater than or equal to two weeks apart.

Prevent Transmission

In areas where the disease is widespread (map page 3), contact with contaminated soil or water can put people at risk for melioidosis. However, in these areas, there are things that certain groups of people can do to help minimize the risk of exposure:

Basic hygiene

Thoroughly clean skin lacerations, abrasions, or burns that have been contaminated with soil or surface water.

Persons with open skin wounds and those with diabetes or chronic renal disease are at increased risk for melioidosis and should avoid contact with soil and standing water.

Those who perform agricultural work should wear boots, which can prevent infection through the feet and lower legs.

Vaccination

No vaccines are licensed for the prevention of melioidosis.

Post-exposure prophylaxis

After exposure to *B. pseudomallei* (particularly following a laboratory accident) combined treatment with co-trimoxazole and doxycycline is recommended. Trovafloxacin and grepafloxacin have been shown to be effective in animal models.

Health care precaution and isolation

Health care workers can use standard contact precautions (mask, gloves, and gown) to help prevent infection.