Chapter 9
MELIOIDOSIS

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INTRODUCTION

In 1911, Captain Alfred Whitmore and Dr CS Krishnaswami described a previously unrecognized disease that was prevalent among the ill-nourished and neglected inhabitants of Rangoon, Burma. The new disease resembled glanders, a zoonotic disease of equines, and the gram-negative bacillus they isolated post-mortem from tissue samples resembled the glanders bacillus, Bacillus mallei. However, the new bacillus could be differentiated from B mallei by its motility, luxuriant growth on peptone agar, and wrinkled colony morphology, and was subsequently named Bacillus pseudomallei. Whitmore’s detailed account of the first 38 human cases of this disease demonstrated most were morphine injectors that died of septicemia with abscesses in multiple organs. As a result, the disease became known as “Whitmore’s disease” or “morphine Injector’s septicemia.” In 1921, Stanton and Fletcher reported an outbreak of a septicemic disease in a guinea pig colony at the Institute for Medical Research in Kuala Lumpur. The infectious agent they isolated from diseased animals was indistinguishable from Whitmore’s bacillus, and they coined the term “melioidosis” (a Greek term meaning glanders-like illness) to describe this new disease of the tropics. Stanton and Fletcher subsequently published a classic monograph in 1932 describing their observations of melioidosis in humans and animals occurring in Burma, Malaya, French Indochina, and Ceylon over a number of years.

Today, melioidosis is regarded as an emerging infectious disease and a potential bioterrorism threat. The etiologic agent of melioidosis is present in water and soil in tropical and subtropical regions and is spread to humans through direct contact with the contaminated source. Clinical manifestations range from subclinical infection to overwhelming septicemia that resembles disseminated or localized, suppurative infection due to a variety of pathogens, resulting in the nickname “the remarkable imitator.” The majority of melioidosis cases have one or more identified risk factors, including diabetes, alcoholism, chronic renal disease, cystic fibrosis, and steroid abuse. Interestingly, acquired immunodeficiency syndrome does not seem to be a major risk factor for melioidosis. Healthy individuals can also get the disease, especially if they work in muddy soil without good hand and foot protection. Many animal species are susceptible to melioidosis, including sheep, goats, horses, swine, cattle, dogs, and cats. Numerous review articles on melioidosis have been published since 1990.

THE INFECTIOUS AGENT

The bacterium that causes melioidosis, now designated Burkholderia pseudomallei, has undergone numerous name changes since its original classification as Bacillus pseudomallei, including Bacterium whitmori, Bacillus whitmori, Pfeifferella whitmori, Pfeifferella pseudomallei, Actinobacillus pseudomallei, Lofflerella whitmori, Flavobacterium pseudomallei, Malleomyces pseudomallei, and Pseudomonas pseudomallei. The non-sporulating, gram-negative bacillus is an environmental saprophyte found in surface waters and wet soils in endemic regions. Individual cells are approximately 0.8 x 1.5 µm with a polar tuft of two to four flagella and may exhibit bipolar staining with a “safety pin” appearance. B pseudomallei is metabolically versatile and can grow on numerous carbon sources. Anaerobic growth is possible, but only in the presence of nitrate or arginine. The microbe accumulates intracellular stores of poly-β-hydroxybutyric acid and can survive in distilled water for years. The optimal survival temperature for B pseudomallei is between 24°C and 32°C, but it can grow at temperatures up to 42°C. B pseudomallei demonstrate considerable interstrain and medium-dependent colony morphology. The oxidase-positive organism can grow on a variety of microbial media, but Ashdown’s selective medium is often used for isolating B pseudomallei from environmental and clinical specimens. Two distinct colony phenotypes are commonly observed on this medium (Figure 9-1a), presumably due to the differential uptake of crystal violet and neutral red or to the differential production of ammonia and oxalic acid. Most strains appear lavender after 2 to 3 days of incubation at 37°C, but some isolates appear deep purple (see Figure 9-1a). After 3 days at 37°C, the colonies often become dull and wrinkled (Figure 9-1b) and emit a distinctive sweet, earthy smell. Other selective media have also been used to isolate B pseudomallei from contaminated specimens.

The complete genome sequence of B pseudomallei K96243, a strain isolated in 1996 from a 34-year-old diabetic patient in Khon Kaen, Thailand, was published in 2004. The 7.25-megabase pair (Mb) genome was comprised of two circular replicons, termed chromosome 1 (4.07 Mb) and chromosome 2 (3.17 Mb). The G + C content of the genome is 68% and is predicted to encode 5,855 proteins. Chromosome 1 encoded a high proportion of core housekeeping functions (DNA replication, transcription, translation, amino acid and nucleotide metabolism, basic carbohydrate metabolism, and cofactor synthesis), while chromosome 2 encoded...
Figure 9-1. *Burkholderia pseudomallei* colony morphologies as demonstrated on Ashdown’s selective medium\(^5\) supplemented with 100 µg/mL streptomycin. Plates were incubated for 3 days at 37°C (a) and 5 days at 37°C (b).

a high proportion of accessory functions (adaptation to atypical conditions, osmotic protection, and secondary metabolism).\(^{5,6,7}\) Plasmid-like replication genes and accessory genes on chromosome 2 suggest it may have been derived from a plasmid (or megaplasmid) that became an indispensable replicon by acquiring essential functions such as transfer ribonucleic acid genes, amino acid biosynthesis genes, and energy metabolism genes. There are 16 “genomic islands” in the *B. pseudomallei* K96243 genome that appear to have been acquired through horizontal gene transfer.\(^{5,6,7}\) Analysis of 11 additional *B. pseudomallei* strains has led to the identification of at least 128 different genomic islands that are variably present among these strains.\(^{5,6,7}\) Mobile genetic elements, such as prophages, insertion sequences, and integrated plasmids, account for most of the laterally acquired genomic sequences. Recent studies have shown that *B. pseudomallei* strains exhibit significant genomic diversity and that much of the genetic heterogeneity is due to laterally acquired mobile genetic elements.\(^{5,6,7}\) These genomic islands may provide strains that give them a metabolic or virulence advantage over strains that do not contain such sequences. Similarly, autonomously replicating plasmids are variably present in *B. pseudomallei* isolates, but little is known about their biological significance.\(^{5,6,7}\) Complete genome sequences of 13 *B. pseudomallei* isolates (K96243, 1026b, 1106a, 1710b, 668, BPC006, MSHR146, MSHR305, MSHR511, NAU20B-16, NCTC13178, NCTC13179, MSHR520) and draft genome sequences of an additional 54 *B. pseudomallei* isolates are available in GenBank, dramatically enhancing the amount and diversity of genome sequence data available for study of *B. pseudomallei*.\(^{5,6,7}\)

**MILITARY RELEVANCE**

Throughout the 20th century, melioidosis had an impact on the health of soldiers serving in Asia during times of war and peace.\(^{6,6}\) Sporadic melioidosis infections occurred in US and Japanese soldiers during World War II (WWII),\(^{4,5,6,7,8}\) and cases of recrudescent melioidosis in WWII veterans were also reported.\(^{6,7,8}\) During the French Indochina War (1946–1954), there were at least 100 cases of melioidosis in French forces during their fight against the resistance movement led by the Viet Minh.\(^{2,6}\) There were more than 300 cases of melioidosis in US soldiers during the Vietnam War\(^{2}\) and additional cases that did not surface until years after the war’s end, leading to the nickname “Vietnam Time Bomb.”\(^{7,1,2}\) Twenty-three melioidosis cases were reported in the Singapore Armed Forces from 1987 to 1994.\(^{74}\) The infection rate in these relatively healthy Singapore Armed Forces was approximately fourfold the rate in the general population of Singapore, suggesting that close contact with the soil during military training may lead to an increased risk for melioidosis. *B. pseudomallei* is a Centers for Disease Control and Prevention Tier 1 select agent that must be handled in biosafety level 3 laboratories.\(^{9}\) Biosafety level 3 facilities incorporate specialized negative-air pressure ventilation systems and well-defined biosafety containment equipment and protocols to study agents that can be transmitted through the air and cause potentially lethal infection. Tier 1 agents present the greatest risk of deliberate misuse and pose a severe threat to public health and safety. *B. pseudomallei* was studied by the United States, the former Soviet Union, and possibly Egypt as a potential biological warfare agent, but was
never used in this capacity. On the other hand, _B. mallei_ was used as a biological warfare agent several times in the past, including during the American Civil War, World War I, World War II, and in Afghanistan between 1982 and 1984. The usefulness of _B. pseudomallei_ as a biological warfare agent is currently unknown, but the ease of acquiring strains from the environment, the ability to genetically manipulate the agent to be multiply antibiotic resistant, and the lack of a melioidosis vaccine make this possibility a serious concern.

### THE DISEASE

#### Epidemiology

Melioidosis cases are most commonly reported from countries located between 20°N and 20°S in latitude, with the greatest concentration in Vietnam, Cambodia, Laos, Thailand, Malaysia, Singapore, and northern Australia. The disease has also been observed in the South Pacific, Africa, India, and the Middle East. In addition, sporadic cases of melioidosis have occurred in the Western Hemisphere in Aruba, Brazil, Mexico, Panama, Ecuador, Haiti, Peru, and Guyana. In endemic regions, the disease occurs in humans, sheep, goats, horses, swine, cattle, dogs, cats, deer, camels, monkeys, zebras, kangaroos, koalas, birds, and crocodiles. Melioidosis cases that occur in temperate regions are typically acquired in endemic areas. Human cases can often be attributed to recent travel to such areas. For example, the first case report of cerebral melioidosis in the United States was recently described in a individual that had immigrated from Cambodia and frequently visited his home country. A case of neurological melioidosis was reported in a pigtail macaque that was imported from Indonesia to the United States for research purposes. In addition, _B. pseudomallei_ infections have been described in pet green iguanas in California and, based on the multilocus sequence typing of the isolates in these cases, are believed to have originated in Central America. With the exception of a single case of melioidosis that occurred in Arizona in 2008, no _B. pseudomallei_ infections have been documented in US patients without a history of prior travel to an endemic region. While the source of the Arizona isolate could not be determined despite extensive investigation, molecular analysis indicated that it was consistent with Southeast Asian origin.

#### Pathogenesis

Several animal models of melioidosis have been developed to study pathogenesis, virulence factors, and efficacy of antibiotics and vaccines. In general, hamsters and ferrets are highly susceptible to experimental melioidosis, while rats, pigs, and rhesus monkeys are relatively resistant. Infant rats can be made more susceptible to infection by intraperitoneal injection of streptozotocin, a compound that induces diabetes. The LD₅₀ (amount necessary to kill 50% of the subject population) of _B. pseudomallei_ for non-diabetic infant rats is greater than 10⁷ bacteria, while the LD₅₀ in streptozotocin-induced diabetic infant rats is approximately 10⁴ bacteria. Mice and guinea pigs exhibit intermediate susceptibility to experimental infection with _B. pseudomallei_, but the LD₅₀ for mice varies widely depending on the route of infection, mouse strain, and bacterial strain. Recent reviews describe in detail the various mouse models of melioidosis that have been developed and the advantages and disadvantages of each.

Due to fears that _B. pseudomallei_ might be used as a biological weapon, basic research on this pathogen has progressed rapidly over the past 10 years. The identification of virulence factors has been facilitated by the availability of genomic sequence data and the existence of a nonpathogenic _B. pseudomallei_-like species designated _B. thailandensis_. _B. pseudomallei_ and _B. thailandensis_ strains are genetically and immunologically similar to one another, but _B. thailandensis_ is less virulent in animal models of infection and has only rarely been reported to cause disease in humans. Genetic determinants that confer enhanced virulence in _B. pseudomallei_ relative to _B. thailandensis_ have been identified by comparative analysis of genomic DNA from these species. Table 9-1 provides a brief description of _B. pseudomallei_ virulence factors identified to date, their mechanisms of action, and their relative importance in animal models of melioidosis.

_B. pseudomallei_ is a facultative intracellular pathogen whose ability to survive and replicate in phagocytic and nonphagocytic cell lines has been well documented. The organism possesses a variety of mechanisms to adapt to the host environment and harbors an array of specialized secretory systems, which are required within this niche. Although relatively little is known about the initial interactions of _B. pseudomallei_ with host cells, recent studies have identified a number of adhesins that mediate adherence of the organism to eukaryotic cells. Following internalization, _B. pseudomallei_ rapidly escapes from endocytic vacuoles and enters into the host cell cytosol, where it can replicate to high numbers, polymerize actin, and induce host cell fusion. These processes are believed...
to facilitate intracellular spread and multinucleated giant cell formation. Type III and type VI secretions systems have been shown to play key roles in endosomal escape and multinucleated giant cell formation, respectively.124,134 The effector proteins delivered by these systems are predicted to modulate critical host proteins and pathways involved in cytoskeleton re-arrangement, cell signaling, and cell death, thereby enabling pathogen survival and proliferation within a host.17 It has been postulated that after the initial phase of infection, B pseudomallei can persist in a dormant stage in macrophages for months or years.123 Melioidosis has the potential for a long latency period and B pseudomallei’s intracellular persistence could provide a mechanism by which this occurs. Intracellular survival and cell-to-cell spread may also provide B pseudomallei protection from the humoral immune response.

Clinical Disease

Melioidosis is a tropical bacterial disease with primary endemic foci in southeast Asia, northern Australia, south Asia, and China. Hyperendemic areas for melioidosis include northern Australia and northeast Thailand, where the disease incidence peaks in the rainy season. The routes of infection include percutaneous inoculation, inhalation, or ingestion of contaminated food or water. Although percutaneous inoculation is the most common route of infection, heavy rainfall is associated with pneumonia and more severe disease and may represent a shift from percutaneous inoculation to inhalation as the primary mode of infection.135 In hyperendemic areas, B pseudomallei causes a substantial burden of infectious disease. For example, at the Sappasit Prasong Hospital in Ubon Ratchatani, northeast Thailand, which serves a rural community of rice farmers and their families, nearly 20% of all community-acquired bacteremia is due to B pseudomallei.136 Likewise, melioidosis is the most common cause of fatal community-acquired bacteremic pneumonia at the Royal Darwin Hospital in the Northern Territory of Australia.137

Cases of human-to-human transmission of B pseudomallei, although very rare, have been documented138,139 The incubation period (time between exposure and appearance of clinical symptoms) varies, as infectious dose, route of infection, B pseudomallei strain characteristics, and host risk factors are all believed to play an important role. One study that looked at the incubation period after inoculation exposures in Darwin, Australia, revealed a mean incubation period of 9 days, with a range of 1 to 21 days.136 Although serologic studies suggest that most infections with B pseudomallei are asymptomatic,141 individuals with risk factors such as diabetes mellitus, alcoholism, cirrhosis, thalassemia, or other immunosuppressed states are at an increased risk of developing symptomatic infection. Other melioidosis-associated risk factors include chronic lung disease, kava consumption, and cystic fibrosis. Diabetes appears to be the most important of all the known risk factors, as up to 50% of patients with melioidosis have diabetes mellitus.27

Melioidosis, which presents as a febrile illness, has an unusually broad range of clinical presentations. The diversity of infectious presentations includes acute localized suppurative soft tissue infections, acute pulmonary infections, acute fulminant septicemia, and chronic localized infections. Clinical disease with B pseudomallei is generally caused by hematogenous spread of bacteria and seeding to various organs within the host.27 The Infectious Disease Association of Thailand, the country with the largest number of reported cases (2,000–3,000 per year), divided 345 cases into the following categories: (a) disseminated septicemia, 45% of the cases with 87% mortality; (b) nondisseminated septicemia, 12% of the cases with 17% mortality; (c) localized septicemia, 42% of the cases with 9% mortality; and (d) transient bacteremia, 0.3% of cases.142,143

Melioidosis is characterized by abscess formation, and the majority of patients with melioidosis are bacteremic. The most commonly involved organ is the lung. The nidus of infection is either a primary pneumonia or lung abscess, or the infection results from hematogenous seeding of the lung from bacteremia (Figures 9-2 and 9-3). For example, of the 540 cases of melioidosis analyzed in the 20-year Darwin Prospective Melioidosis Study, pneumonia was the most common primary

Figure 9-2. Chest radiograph demonstrating a severe multilobar pneumonia. Photograph courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.
TABLE 9-1
CANDIDATE VIRULENCE FACTORS OF BURKHOLDERIA PSEUDOMALLEI

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
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<tbody>
<tr>
<td>Capsular polysaccharide</td>
<td>CPS is a 200-kDa 1,3-linked 2-O-acetyl-6-deoxy-β-D-manno-heptan. CPS mutants are highly attenuated in hamsters and mice. CPS may contribute to survival in serum by reducing complement factor C3b deposition.</td>
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<tr>
<td>Type III secretion system</td>
<td><em>B. pseudomallei</em> harbors three distinct TTSS loci, termed TTSS1, TTSS2, and TTSS3. The TTSS1 and TTSS2 loci are similar to TTSS genes of the plant pathogen <em>Ralstonia solanacearum</em> and are not necessary for virulence in hamsters. The TTSS3 locus is similar to the TTSS in <em>Salmonella</em> and <em>Shigella</em> and is required for full virulence of <em>B. pseudomallei</em> in both hamsters and mice. The effector proteins of TTSS3 facilitate the invasion of epithelial cells and escape from endocytic vesicles.</td>
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<tr>
<td>Type VI secretion system</td>
<td><em>B. pseudomallei</em> harbors six distinct T6SS loci designated T6SS-1, T6SS-2, T6SS-3, T6SS-4, T6SS-5, and T6SS-6. Of the six systems, only T6SS-1 has been shown to be necessary for virulence in animal models of melioidosis. This system is expressed following uptake by murine macrophages (RAW264.7 cells) and is required for optimal intracellular growth, actin-based motility and multinucleated giant cell formation.</td>
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<tr>
<td>Transcriptional regulators and two-component regulatory systems</td>
<td><em>B. pseudomallei</em> harbors numerous transcriptional regulators and two component regulatory systems. Bspk (TetR family regulator), bsaN, and bprC (AraC family regulators) are involved in a complex regulatory cascade that ultimately controls TTSS and T6SS-1 gene expression. B pseudomallei bspR, bsaN, and bprC mutants exhibited significantly attenuated virulence in mice. The VirAG two-component regulatory system is known to positively regulate T6SS-1 gene expression, and mutations in VirAG render the organism avirulent in mice.</td>
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<tr>
<td>Quorum sensing</td>
<td><em>B. pseudomallei</em> encodes three <em>luxI</em> homologues that produce at least three quorum-sensing molecules, including N-octanoyl-homoserine lactone (C8-HSL), N-decanoyl-homoserine lactone (C10-HSL), and N-(3-hydroxyoctanoyl)-L-homoserine lactone (3-hydroxy-C8-HSL). It also has five <em>luxR</em> homologs to sense these signals. Mutations in all of the <em>luxI</em> and <em>luxR</em> homologues result in strains with decreased virulence in hamsters and mice, but the virulence-associated genes regulated by this complex quorum-sensing system are still under investigation.</td>
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<tr>
<td>Lipopolysaccharide O-antigen</td>
<td>An unbranched heteropolymer consisting of disaccharide repeats having the structure -(3)-β-D-glucopyranose-(1-3)-6-deoxy-α-L-talopyranose-(1-, in which the 6-deoxy-α-L-talopyranose residues possess 2-O-acetyl or 2-O-methyl and 4-O-acetyl modifications. LPS O-antigen mutants are attenuated in hamsters, guinea pigs, and infant diabetic rats and are killed by serum. This factor promotes survival in serum by preventing killing by the alternative pathway of complement. Levels of anti-LPS O-antigen antibodies are significantly higher in patients who survive than in those who die.</td>
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<tr>
<td>Flagellin</td>
<td>A surface-associated 43-kDa protein that is required for motility. Flagellin mutants are attenuated in mice, but not in hamsters or infant diabetic rats. Passive exposure studies demonstrated that flagellin-specific antiserum was capable of protecting infant diabetic rats from challenge with <em>B. pseudomallei</em>.</td>
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<tr>
<td>Type II secretion</td>
<td>Required for the secretion of several exoproducts, including protease, lipase, and phospholipase.</td>
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<tr>
<td>Type IV pili</td>
<td><em>B. pseudomallei</em> K96243 encodes four complete type IV pilin clusters. A mutation in pilA, a gene encoding a type IVA pilin subunit, results in a strain exhibiting decreased attachment to cultured respiratory cell lines relative to wild type. The pilA mutant was not attenuated in mice by the intraperitoneal challenge route, but was slightly attenuated by the intranasal challenge route.</td>
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<tr>
<td>Biofilm formation</td>
<td>The extracellular slime matrix produced by <em>B. pseudomallei</em> appears to be polysaccharide in nature, but the exact structure is unknown. Biofilm mutants were not attenuated in the mouse model of melioidosis, suggesting that the biofilm plays a relatively minor role, if any, in virulence.</td>
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<tr>
<td>Malleobactin</td>
<td>A water-soluble siderophile of the hydroxamate class. The compound is capable of scavenging iron from both lactoferrin and transferrin in vitro. The genes encoding malleobactin biosynthesis and transport were recently identified, but malleobactin mutants were not tested in animal models of melioidosis.</td>
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| Rhamnolipid                            |A 762-Da glycolipid with the structure 2-O-α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxytetradecanoyl-β-hydroxytetradecanoate (Rha-Rha-C14-C14). Rhamnolipid-treated cell lines exhibit profound morphological alterations, but the role of this glycolipid in virulence remains unknown. (Table 9-1 continues)
Table 9-1 continued

| Exopolysaccharide | A linear, unbranched polymer of repeating tetrasaccharide units composed of α-galactose and 3-deoxy-α-manno-octulosonic acid (KDO), with the following structure: –3)-2-O-Ac-β-D-Galp(1-4)-α-D-Galp(1-3)-β-D-Galp(1-5)-β-D-KDOp(2-3,5)-EPS is not produced by the closely related nonpathogenic species *B thailandensis*, suggesting that it may be a virulence determinant of *B pseudomallei*. EPS is probably produced during infection because sera from melioidosis patients contain IgG and IgM antibodies to EPS. 38,40
| Endotoxin | The lipid A portion of *B pseudomallei* LPS contains amide-linked 3-hydroxyhexadecanoic acids, which are longer than the fatty acid chains of enterobacterial LPS. 41 The endotoxic activity of *B pseudomallei* LPS was 10- to 100-fold weaker than enterobacterial LPS in pyrogenic activity in rabbits, lethal toxicity in GalN-sensitized mice, and macrophage activation assays. However, the mitogenic activity of *B pseudomallei* LPS was much higher than enterobacterial LPS. 41 The LD₅₀ of purified *B pseudomallei* LPS in hamsters was 1,000 mg. 42
| Actin-based motility | Once *B pseudomallei* gains access to the host cell cytoplasm, it can replicate and exploit actin-based motility for cell-to-cell spread and evasion of the humoral immune response. 43-45 The autotransported protein *bimA* is located at the pole of the bacterial cell and is responsible for the formation of actin tails. 44 It is currently unknown if actin-based motility is required for virulence in animal models of melioidosis.
| Autotransporters and adhesins | *B pseudomallei* harbors 11 autotransporter analogs, including *bimA*, and two ATs (*boaA* and *boaB*) with roles in bacterial adherence to epithelial cells, eight ATs (*BpaA-F; BcaA-B*) that contribute to adherence to and efficient invasion of A549 cells. 46-48 *BpaC* and *bcaA* have been implicated in virulence in BALB/c mice, since mutants in these ATs displayed defects in dissemination to the liver or spleen, respectively. 47 A *B pseudomallei* *bfaA* (also known as *bpaF*) mutant demonstrated a moderate attenuation in a murine model of melioidosis. 48
| Exotoxins | There have been several reports in the literature about *B pseudomallei* exotoxins, 49-53 but the genes encoding these exotoxins have not been identified and no defined exotoxin mutants have been constructed. The role of exotoxins as *B pseudomallei* virulence factors is highly controversial and there appears to be no correlation between in vitro cytotoxicity and in vivo virulence. 42,54 In fact, the K96243 genome sequence does not encode any homologues of known major toxins produced by other pathogenic bacteria. 29
| Intracellular toxin | *Burkholderia* lethal factor 1 (BLF1; BPSL1549) is structurally related to cytotoxic necrotizing factor 1 and has been shown to specifically deamidate Gln 39 of eukaryotic initiation factor 4A (a translation initiation factor) leading to inhibition of protein synthesis. 55,56 Purified recombinant BLF1 was toxic to mice (via intraperitoneal injection), 774 macrophages, but not 3T3 cells. 58,59 A *B pseudomallei* *bfl1* mutant was significantly attenuated in mice and exhibited a 100-fold higher median lethal dose in comparison to the type strain. 30,56

**Table 9-1 continued**

| CPS: capsular polysaccharide; EPS: exopolysaccharide; Gln: glutamine; IgG: immunoglobulin G; IgM: immunoglobulin M; LPS: lipopolysaccharide; T6SS: type VI secretion system; TTSS: type III secretion system | **Table 9-1 continued**
Table 9-1 continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Authors</th>
<th>Title</th>
<th>Journal</th>
<th>Year</th>
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- **Medical Aspects of Biological Warfare**
- **Clinical presentation, occurring in 278 (51%) of the patients.**
- **Patients with acute pulmonary melioidosis present with cough, fever, sputum production, and respiratory distress, and can present with or without shock.** Chronic pulmonary melioidosis mimics tuberculosis, with side effects including purulent sputum production, cough, hemoptysis, and night sweats.
- **Patients with the acute septic form of melioidosis present characteristically with a short history of fever and no clinical evidence of focal infection.** Most patients are profoundly ill with signs of sepsis. Septic shock may appear on presentation.
- **In the Darwin Prospective Melioidosis Study, 298 (55%) of patients were bacteremic on presentation to the hospital. Septic shock, usually occurring on, or within 24 hours of, admission to the hospital, was associated with 50% mortality, while bactereemia without septic shock was associated with a 7%**
Melioidosis

Figure 9-3. Autopsy specimen demonstrating extensive pulmonary involvement with abscess formation due to *B. pseudomallei*. Photograph courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

Hematogenous seeding and abscess formation can occur in any organ (Figure 9-4); however, liver, spleen, skeletal muscle, prostate, and kidney are the most common abscess sites (Figures 9-5 and 9-6).

Less common presentations of melioidosis include uncomplicated infections of the skin (Figure 9-7), subcutaneous tissues, or the eye. Corneal ulcerations resulting from trauma, which become secondarily infected with *B. pseudomallei*, are rapidly destructive. Septic arthritis and osteomyelitis (Figure 9-8) have also been described, but cellulitis appears to be rare. In a prospective study of more than 2,000 patients with melioidosis in Thailand, primary meningitis or endocarditis was not observed, but meningitis secondary to cerebral abscess rupture and mycotic aneurysms was seen. Other unusual melioidosis presentations include mediastinal masses, pericardial fluid collections, and adrenal abscesses. The clinical presentation of melioidosis also varies among different regions. In Thailand, 30% of the melioidosis cases in children present as acute suppurative parotitis. These children present with fever, pain, and swelling over the parotid (salivary) gland without other evidence of underlying predisposing conditions. In 10% of the cases, the swelling is bilateral. Although acute suppurative parotitis is unusual in Australia, approximately 4% of the melioidosis cases there present as brainstem encephalitis with peripheral motor

Figure 9-4. Pustules with an erythematous base due to septicemic melioidosis. Photograph courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

Figure 9-5. Computed tomography scan showing multiloculated liver abscess. Photograph courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

Figure 9-6. Computer tomography scan showing prostatic abscess. Photograph courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

Figure 9-7. Pustules with an erythematous base due to septicemic melioidosis. Photograph courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.
weakness or flaccid paraparesis. Features associated with this presentation include limb weakness, cerebellar signs, and cranial nerve palsies. Patients with this syndrome usually have an initial normal state of consciousness. Multiple focal *B pseudomallei* micro abscesses in the brainstem and spinal cord are probably responsible for this syndrome.27 Although acute infections in individuals with predisposing risk factors are the most common, latent infection with reactivation, resulting in an illness that can resemble tuberculosis, also occurs with melioidosis. During the Vietnam War, large numbers of Western soldiers were exposed to *B pseudomallei* through inhalation, contaminated wounds, or burns. A serologic survey of US military personnel demonstrated that mild or unapparent infection was common, and estimated that 225,000 people with subclinical infection were potentially at risk for reactivation.146 Fortunately, the number of cases of reactivation melioidosis in these individuals has remained rare compared to the number of individuals exposed. Long latency periods between exposure and development of melioidosis in nonendemic regions have been reported.70 Recently, a case of cutaneous melioidosis in a man taken prisoner by the Japanese during World War II was described. This man is presumed to have had reactivated melioidosis 62 years after exposure, as he had not returned to an area of melioidosis endemicity after being imprisoned in northwest Thailand.39

**Diagnosis**

Because of its protean clinical manifestations, the diagnosis of melioidosis depends on the isolation and identification of *B pseudomallei* from clinical specimens. Melioidosis should be suspected in any severely ill, febrile patient with an associated risk factor who has been in an endemic area. *B pseudomallei* can grow on most routine laboratory media and can be isolated from normally sterile sites, such as blood, by standard techniques.23 The organism is usually detected in blood culture within 48 hours. Isolator centrifugation blood culture systems result in quicker detection times, but are less sensitive compared to conventional broth-based blood culture.147 Ashdown's medium, a crystal violet and gentamicin-containing medium that permits selective growth of *B pseudomallei* (see Figure 9-1), has been used to significantly increase the frequency of recovery of *B pseudomallei* from the rectum, wounds, and sputum compared to recovery on blood and MacConkey agars.50 Patients with suspected melioidosis should submit blood, sputum, urine, and abscess fluid, as well as throat wound and rectal swabs for culture.

*B pseudomallei* is intrinsically resistant to aminoglycosides and polymyxins.148,149 This unusual antibiotic profile (gentamicin and colistin resistance, but amoxicillin-clavulanate susceptibility) in an oxidase-positive, gram-negative bacillus is helpful for identifying *B pseudomallei* in the microbiology laboratory. Commercially available kits for bacterial identification, such as the API 20NE (bioMérieux, Marcy l'Etoile, France), have been reported to reliably confirm the identity of *B pseudomallei*,49 although other investigators have reported mixed results.150 The Vitek 1 (bioMérieux) has also been found to be highly sensitive, having identified 99% of the 103 *B pseudomallei* isolates tested.151

![Figure 9-7](image1) Skin lesions associated with melioidosis on the lower extremity. Photograph courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

![Figure 9-8](image2) Chronic osteomyelitis of the lower extremity due to melioidosis. Photograph courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.
However, in this same study, the Vitek 2 (bioMérieux) identified only 19% of these same isolates. Other studies continue to document difficulties in identifying \( B\) pseudomallei with the Vitek 2.\(^{152,153}\) It has also been recently reported that \( B\) pseudomallei specimens from infections that occurred in exotic locations, such as Malaysian Borneo, were misidentified as \( B\) cepacia by the Vitek 2.\(^{154}\)

Serologic testing alone is not a reliable method of diagnosis. An indirect hemagglutination test and other serologic tests may produce false negatives in patients with sepsis, as well as false positives due to a high prevalence of antibodies to \( B\) pseudomallei in healthy individuals from endemic areas.\(^{143}\) A recently published paper from Australia proposed a highly sensitive \( B\) pseudomallei identification algorithm that makes use of screening tests (Gram stain, oxidase test, gentamicin, and polymyxin susceptibility testing) combined with monoclonal antibody agglutination testing and gas-liquid chromatography analysis of bacterial fatty acid methyl esters.\(^{155}\) Various polymerase-chain-reaction–based identification techniques have also been developed to aid in the identification of \( B\) pseudomallei.\(^{156,157}\) A recent comparison of the sensitivities and specificities of seven different real-time TaqMan (Life Technologies, Grand Island, New York) polymerase chain reaction assays for detecting \( B\) pseudomallei demonstrated that an assay targeting the type III secretion system (TTS1-orf2) performed the best at detecting \( B\) pseudomallei directly from clinical samples.\(^{158}\)

**Treatment**

Asymptomatic carriage of \( B\) pseudomallei appears to be a very rare event\(^{159}\); therefore, the isolation of \( B\) pseudomallei from a clinical specimen indicates that treatment is required. Melioidosis requires prolonged antibiotic therapy to cure the infection and prevent relapse. Melioidosis cases should be treated with initial intensive therapy (at least 2 weeks of intravenous [IV] therapy) followed by oral eradication therapy for a minimum of 3 months. Cases presenting with localized mild disease can be treated with oral eradication therapy without initial parenteral treatment. The choice of therapy for treating melioidosis is complicated because \( B\) pseudomallei is resistant to many antibiotics, including penicillin, ampicillin, aminoglycosides, first- and second-generation cephalosporins, and colistin.\(^{160,161}\) \( B\) pseudomallei is also relatively insensitive to quinolones and macrolides\(^{162}\); therefore, therapeutic options are limited.

The first study demonstrating the effectiveness of ceftazidime for severe melioidosis was published in 1989. In this study, ceftazidime treatment (120 mg/kg/day) was associated with a reduction of overall mortality from 74% to 37% (\( P = 0.009\)) when compared to “conventional therapy” with chloramphenicol (100 mg/kg/day), doxycycline (4 mg/kg/day), trimethoprim (TMP; 10 mg/kg/day), and sulfamethoxazole (SMX; 50 mg/kg/day).\(^{163}\) In 1992, a second randomized clinical trial of treatment of severe melioidosis conducted in Thailand also demonstrated a substantial reduction in mortality when ceftazidime plus TMP-SMX was used, as compared to the four-drug conventional therapy.\(^{164}\)

In 1999, a comparative treatment trial in Thailand found that imipenem/cilastatin was as effective as ceftazidime for the treatment of severe melioidosis. Although there was no difference in mortality, fewer treatment failures were observed in the patients given imipenem/cilastatin as compared to the ceftazidime group.\(^{165}\) Therefore, initial intensive therapy for melioidosis should consist of high doses of ceftazidime (50 mg/kg, up to 2 g IV every 6 hours), imipenem/cilastatin (25 mg/kg, up to 1 g IV every 6 hours), or meropenem (25 mg/kg, up to 1 g IV every 8 hours) combined with TMP-SMX (320 mg/g/1,600 mg IV or by mouth every 12 hours) for patients with severe infection involving the brain, prostate, or other privileged site, for at least 14 days.\(^{166}\) Critically ill patients with extensive pulmonary disease, organ abscesses, osteomyelitis, septic arthritis, or neurological melioidosis require longer intensive IV therapy (4 weeks or even longer).

The benefit of adding TMP-SMX to the initial antimicrobial regimen is supported by animal data and expert opinion.\(^{26}\) However, a 2005 paper from Thailand, which described two randomized controlled trials comparing ceftazidime alone versus ceftazidime combined with TMP-SMX for severe melioidosis, failed to demonstrate a mortality benefit associated with the addition of TMP-SMX, although the dose of TMP-SMX used in this study appears to be lower than that used in Australia.\(^{167}\) Nonetheless, patients in the Royal Darwin Hospital in the Northern Territory of Australia with severe melioidosis involving the brain, bone, prostate or other sequestered site, or severe pulmonary disease are treated with meropenem and TMP-SMX (Bart Currie, MD, Royal Darwin Hospital, Australia, written communication, April 2013). Meropenem is used rather than imipenem/cilastatin because it has fewer neurological side effects.\(^{162}\)

The median time to resolution of fever is 9 days, but patients with large abscesses or empyema often have fluctuating fevers lasting a month or more. In a 10-year prospective review of 252 melioidosis cases in Australia, internal organ abscesses were common, with the largest majority found in the prostate. Although other internal collections frequently resolve with medical therapy, prostatic abscesses usually require drainage to prevent treatment failures.\(^{137}\)
Adjunctive therapy with recombinant granulocyte colony-stimulating factor (G-CSF) is routinely used for melioidosis patients with septic shock in the Northern Territory of Australia. A retrospective review of mortality rates before and after the addition of G-CSF therapy at the Royal Darwin Hospital was recently published. In this study, the introduction of G-CSF as adjunctive therapy for patients with septic shock was associated with a decrease in mortality from 95% to 10%. However, a randomized controlled clinical trial of G-CSF for treating melioidosis sepsis in Thailand failed to demonstrate a significant mortality benefit to the group that received it in addition to cefazidime. The authors pointed out that the resource-constrained environment where the trial took place (limited ventilator and inotropic support, no invasive monitoring, no dialysis) may have introduced confounding variables.169

After initial intensive therapy, oral maintenance therapy is given for another 12 to 20 weeks to prevent disease relapse.13 Oral maintenance therapy traditionally consists of chloramphenicol (40 mg/kg/day), doxycycline (4 mg/kg/day), and TMP-SMX (10 mg/50 mg/kg/day).170 However, this combination frequently causes side effects that result in problems with compliance. Some experts recommend high-dose TMP-SMX (8 mg/40 mg/kg, up to 320/1,600 mg, by mouth twice daily) combined with doxycycline.142 The combination of TMP-SMX with doxycycline was recently shown to be as effective and better tolerated than the conventional four-drug regimen (chloramphenicol, doxycycline, and TMP-SMX) for maintenance therapy in an open-labeled randomized trial conducted in Thailand.171 In this study, failure to complete at least 12 weeks of maintenance therapy was the most important determinant of relapse. In the Northern Territory of Australia, TMP-SMX (8 mg/40 mg/kg) given every 12 hours is used as monotherapy for maintenance for at least 3 to 6 months, with a low relapse rate (1 failure in fewer than 60 patients).13,137 It should be noted, however, that improvements in rates of relapse, from 6.4% (prior to September 2003) to 1.2% (after September 2003) in the Darwin Prospective Melioidosis Study have been attributed to the improved use of efficacious antimicrobials as well as a lengthened IV treatment phase for complex cases.172 A recent, multicenter, double-blind, randomized placebo-controlled trial comparing the efficacy of 20 weeks of TMP-SMX plus placebo to TMP-SMX plus doxycycline for oral maintenance therapy was recently published. This trial, which enrolled 626 patients from five hospitals in northeast Thailand, demonstrated that TMP-SMX was noninferior to TMP-SMX plus doxycycline for the oral phase of melioidosis treatment. Adverse drug reactions were less common in the TMP-STX group than in the TMP-SMX plus doxycycline group, suggesting that TMP-STX monotherapy is preferred on the basis of safety and patient tolerance.173

Although evidence suggests that it is associated with a higher rate of relapse, amoxicillin-clavulanate can be used for oral maintenance therapy in individuals with a sulfonamide allergy or in pregnant patients. The recommended dose of oral amoxicillin-clavulanate is 20 mg/5 mg/kg, three times a day—a dose of amoxicillin-clavulanate that is higher than usually prescribed.174

Quinolone antibiotics are not recommended as therapy for eradicating B pseudomallei. Ciprofloxacin and ofloxacin were found inferior, with a failure rate of 29%, when compared to a 20-week course of maintenance therapy consisting of amoxicillin-clavulanate or the combination of chloramphenicol, doxycycline, and TMP-SMX.175 Another study also found that the combination of ciprofloxacin plus azithromycin was associated with an unacceptably high rate of relapse.176

Prevention

Several experimental melioidosis vaccines have been tested in rodent models of infection, including live attenuated vaccines, heterologous vaccines, acellular vaccines, and subunit vaccines.177–180 Variability in vaccination protocols, routes of challenge, and animal models make it difficult to directly compare the experimental melioidosis vaccine studies published. In general, most vaccine candidates provided significant protection compared to unvaccinated controls, but none resulted in 100% protection and sterilizing immunity. Several recent comprehensive reviews thoroughly describe the melioidosis vaccine candidates that have been developed to date.178–180

Live attenuated vaccines have been shown to be immunogenic and protective against a variety of facultative intracellular pathogens, including Mycobacterium tuberculosis, Shigella, Salmonella, Yersinia, Listeria monocytogenes, Francisella tularensis, and Brucella melitensis.181–185 B pseudomallei purine auxotrophic mutants generated by ultraviolet and chemical mutagenesis were highly attenuated in mice and provided significant protection against subsequent challenge with virulent strains.186,187 Unfortunately, the molecular nature of the purine-dependent mutations in these strains was unknown, and the possibility of reversion to wild-type could not be eliminated. A B pseudomallei temperature-sensitive mutant (chemically induced) and a branched-chain amino acid auxotroph (transposon mutant) were also tested as live attenuated vaccines and provided significant protection in mice against challenge with virulent strains.186,188 Vaccination of mice with an attenuated strain harboring...
a suicide plasmid disruption of bipD, a gene encoding a type III secretion system translocation protein, resulted in partial protection against challenge with wild-type B pseudomallei. More recently, a highly attenuated B pseudomallei purM mutant (strain Bp82) was evaluated as a live attenuated vaccine and shown to provide mice significant protection against an intranasal challenge with wild-type B pseudomallei. These studies suggest that live attenuated vaccines are promising candidates for melioidosis vaccines; however, strains with defined deletion mutations would be preferred to prevent the possibility of reversion to wild-type.

Iliukhin et al vaccinated guinea pigs with live B thailandensis strains and protected less than 50% of the animals challenged with 200 times the LD₅₀ of wild-type B pseudomallei. B thailandensis and B pseudomallei produce identical lipopolysaccharide (LPS)-O-antigens and contain immunologically related secreted and cell-associated antigens, which probably account for the protection that B thailandensis affords. The B pseudomallei exopolysaccharide and capsular polysaccharide (CPS; see Table 9-1) are not produced by most B thailandensis strains, and both polysaccharides may be necessary for full protection against challenge with B pseudomallei. Recently, B thailandensis strains (eg, E555) that express the CPS have been identified and tested as vaccine candidates. A study by Scott et al showed that immunization with E555 conferred significant protection against a lethal intraperitoneal challenge of B pseudomallei in mice. Live attenuated F tularensis strains were also tested as heterologous vaccine candidates against melioidosis in rodents. Attenuated F tularensis strains did afford some protection against challenge with virulent B pseudomallei.

A crude acellular melioidosis vaccine was produced to protect captive cetaceans at Ocean Park in Hong Kong. The vaccine consisted of a protein-poly saccharide mixture (1:3), and it significantly protected hamsters against experimental challenge with virulent B pseudomallei. The acellular vaccine reduced melioidosis mortality in cetaceans from 45% to less than 1%. Unfortunately, the exact chemical components of the vaccine were not well characterized, leaving a high probability of lot-to-lot variation. A naturally derived outer membrane vesicle vaccine has been developed and tested in BALB/c mice and shown to provide significant protection against a lethal B pseudomallei aerosol challenge. In addition, studies have described the testing of purified protein antigens, including LoIC (ABC transporter protein), PoF (periplasmic binding protein), OppA (oligopeptide-binding protein), BimA (autotransporter protein), BopA (T3SS effector protein), and Hcp1 (T6SS-1 component) as potential vaccine candidates. Significant protection was conferred by several of these protein antigens; sterilizing immunity was not.

In a study by Nelson et al, mice were actively immunized with purified B pseudomallei CPS or LPS and challenged with virulent B pseudomallei by the intraperitoneal or aerosol route. The LPS-vaccinated mice exhibited an increased mean time to death relative to controls, and 50% of the mice survived for 35 days after intraperitoneal challenge. By comparison, mice vaccinated with the purified CPS had an increased mean time to death, but 100% of the vaccinated mice were dead by day 28. Neither of the subunit vaccines provided substantial protection against a lethal aerosol challenge, probably because B pseudomallei appears to be more virulent by this route of infection. Improved subunit vaccines that generate both humoral and cell-mediated immune responses are probably necessary to protect against infection with B pseudomallei.

Several studies have shown that CPS- and LPS-specific monoclonal antibodies (mAbs) can passively protect animals against challenge with B pseudomallei. The bactericidal and opsonophagocytic activities associated with various anti-LPS or anti-CPS mAbs correlated with their protective capacity in mice. CPS and LPS have also been shown to be major components of an outer membrane vesicle vaccine that provided significant protection in mice. Taken together, such findings indicate that these surface-exposed carbohydrates are protective antigens and support the rationale for developing LPS- and CPS-based vaccines for immunization against melioidosis. Both CPS- and OPS-based glycoconjugates have been produced and shown to be capable of eliciting high-titer, carbohydrate-specific antibody responses; however, the protective capacity of these subunit vaccines remains to be reported.

There is no licensed vaccine available to prevent human melioidosis and no definitive evidence that infection with B pseudomallei confers immunity, because reinfection with a different strain of B pseudomallei has occurred after successful melioidosis treatment. The only proven method of disease prevention for individuals with known risk factors is avoiding B pseudomallei in the environment. Recommendations for postexposure prophylaxis (PEP) following a laboratory exposure or bioterrorism event involving B pseudomallei are complicated by the lack of efficacy data from either clinical studies or animal experiments. Recommendations are therefore based on expert consensus opinion from physicians who frequently treat melioidosis cases and with data extrapolated from clinical studies on oral
maintenance therapy. The recommended antibiotic for PEP of \( B \) _pseudomallei_ exposure is TMP-SMX. Dosing is as follows:

- adults greater than 60 kg: 2 × 160 mg/800 mg tablets every 12 hours;
- adults between 40 and 60 kg: 3 × 80 mg/400 mg tablets every 12 hours;
- adults less than 40 kg: 2 × 80 mg/400 mg tablets every 12 hours; and
- children: 8 mg/40 mg/kg, maximum dose 320 mg/1,600 mg, every 12 hours.

If the organism is resistant to TMP-SMX or the patient is unable to take sulfa drugs, amoxicillin-clavulanate is the second choice. Dosing is as follows:

- adults greater than 60 kg: 3 × 500 mg/125 mg tablets every 8 hours;
- adults less than 60 kg: 2 × 500 mg/125 mg tablets every 8 hours; and
- children: 20 mg/5 mg/kg, maximum dose of 1,000 mg/250 mg every 8 hours.\(^{166,208}\)

The recommended duration of PEP is 21 days.

**SUMMARY**

Melioidosis, a disease caused by the saprophytic gram-negative bacterium \( B \) _pseudomallei_, is regarded as an emerging infectious disease and a potential bioterrorism threat. \( B \) _pseudomallei_ is present in water and soil samples in endemic tropical and sub tropical regions, and is spread to humans through percutaneous inoculation from a contaminated source or through inhalation or ingestion. The majority of individuals who develop melioidosis have an identifiable risk factor, such as diabetes mellitus, alcoholism, cirrhosis, or other immunosuppressed state, although healthy people may also develop disease. The incubation period is not clearly defined, but may range from 1 to 21 days. Exposed individuals with subclinical infection are potentially at risk for reactivation, which can occur many years later.

Melioidosis has an unusually broad range of clinical presentations. Clinical disease is generally caused by hematogenous seeding of bacteria to various organs within the host, resulting in abscess formation. The majority of patients with melioidosis present to the hospital with bacteremia. Because of its protean clinical manifestations, the diagnosis of melioidosis depends on the isolation and identification of \( B \) _pseudomallei_ from clinical specimens. Ashdown’s selective medium is often used to increase the recovery of \( B \) _pseudomallei_ from nonsterile clinical specimens. Serologic testing alone is not a reliable method of diagnosis because there is a high prevalence of antibodies to \( B \) _pseudomallei_ in healthy individuals in endemic areas, and false negative results in patients with sepsis.

All melioidosis cases should be treated with initial intensive therapy followed by oral eradication therapy. \( B \) _pseudomallei_ is inherently resistant to many antibiotics, which complicates therapeutic decisions. Antibiotics recommended to treat melioidosis are ceftazidime, imipenem/cilastatin, meropenem, and TMP-SMX.

Various experimental melioidosis vaccines have been tested in animal models, but no licensed vaccine exists to prevent human infections. Avoidance of \( B \) _pseudomallei_ by individuals with known risk factors is the only proven method of disease prevention. The efficacy of PEP in preventing human disease after exposure is unknown, although guidelines based on expert opinion have been published.

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Melioidosis


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