Chapter 7

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.[1] The new disease resembled glanders, a zoonotic disease of equines.[2] Whitmore and Krishnaswami isolated a gram-negative bacillus that resembled the glanders bacillus, *Bacillus mallei*, from postmortem tissue samples.[3] However, the new bacillus could be differentiated from *B mallei* by its motility, luxuriant growth on peptone agar, and wrinkled colony morphology; it was subsequently named *Bacillus pseudomallei*.[3,4] Whitmore's detailed account of the first 38 human cases of this disease demonstrated that most of those affected were morphine injectors who died of septicemia with abscesses in multiple organs.[4] As a result, the disease became known as "Whitmore's disease" or "morphine injector's septicemia."[5,6] 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.[7] Stanton and Fletcher isolated an infectious agent from diseased animals that was indistinguishable from Whitmore's bacillus, and they named it "melioidosis" (a Greek term meaning glanders-like illness) to describe this new disease of the tropics.[7] 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.[8]

Melioidosis is regarded as an emerging infectious disease and a potential bioterrorism threat.[9-11] The etiologic agent of melioidosis is present in water and soil in tropical and subtropical regions; it 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 attributable to a variety of pathogens, resulting in the nickname "the remarkable imitator."[12] The majority of melioidosis cases have identified risk factors, including diabetes, alcoholism, chronic renal disease, cystic fibrosis, and steroid abuse.[13] AIDS does not seem to be a major risk factor for melioidosis. Healthy individuals can also contract melioidosis, especially if they work in muddy soil without good hand and foot protection.[14] Many animal species are susceptible to melioidosis, including sheep, goats, horses, swine, cattle, dogs, and cats.[15] Numerous review articles on melioidosis have been published since 1990.[11,13-27]

INFECTIOUS AGENT

The bacterium that causes melioidosis, now designated *Burkholderia pseudomallei*,[28] has undergone numerous name changes since its original classification as *B pseudomallei*, including (*a*) *Bacterium whitmori*, (*b*) *Bacillus whitmori*, (*c*) *Pfeifferella whitmori*, (*d*) *Pfeifferella pseudomallei*, (*e*) *Actinobacillus pseudomallei*, (*f*) *Lofflerella whitmori*, (*g*) *Flavobacterium pseudomallei*, (*h*) *Malleomyces pseudomallei*, and (*i*) *Pseudomonas pseudomallei*. The nonsporulating, gram-negative bacillus is an environmental saprophyte found in surface waters and wet soils in endemic regions.[29-36] Individual cells, which are approximately 0.8 x 1.5 μm, have a polar tuft of two to four flagella and exhibit bipolar staining with a "safety pin" appearance.[37,38] *B pseudomallei* is metabolically versatile and can grow on numerous carbon sources.[28,39] Anaerobic growth is possible, but only in the presence of nitrate or arginine.[11] The microbe accumulates intracellular stores of poly-β-hydroxybutyric acid and can survive in distilled water for years.[10,40,41] The optimal survival temperature for *B pseudomallei* is between 24°C and 32°C, but it can grow at temperatures up to 42°C.[42,43] *B pseudomallei* demonstrates considerable interstrain and medium-dependent colony morphology.[44-46] 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.[47] Two distinct colony phenotypes are commonly observed on this medium (Figure 7-1), probably because of the differential uptake of crystal violet and neutral red or the differential production of ammonia and oxalic acid.[47,48] Most strains appear lavender after 2 to 3 days of incubation at 37°C, but some isolates appear deep purple (see Figure 7-1). After 5 days at 37°C, the colonies often become dull and wrinkled (see Figure 7-1) and emit a distinctive sweet earthy smell. Other selective media have also been used to isolate *B pseudomallei* from contaminated specimens.[49,50]

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 recently determined.[51] The 7.25-megabase pair (Mb) genome was composed of two circular replicons, termed chromosome 1 (4.07 Mb) and chromosome 2 (3.17 Mb). The G + C content of the genome was 68% and 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); and chromosome 2 encoded a high proportion of accessory functions (adaptation to atypical conditions, osmotic protection, and secondary

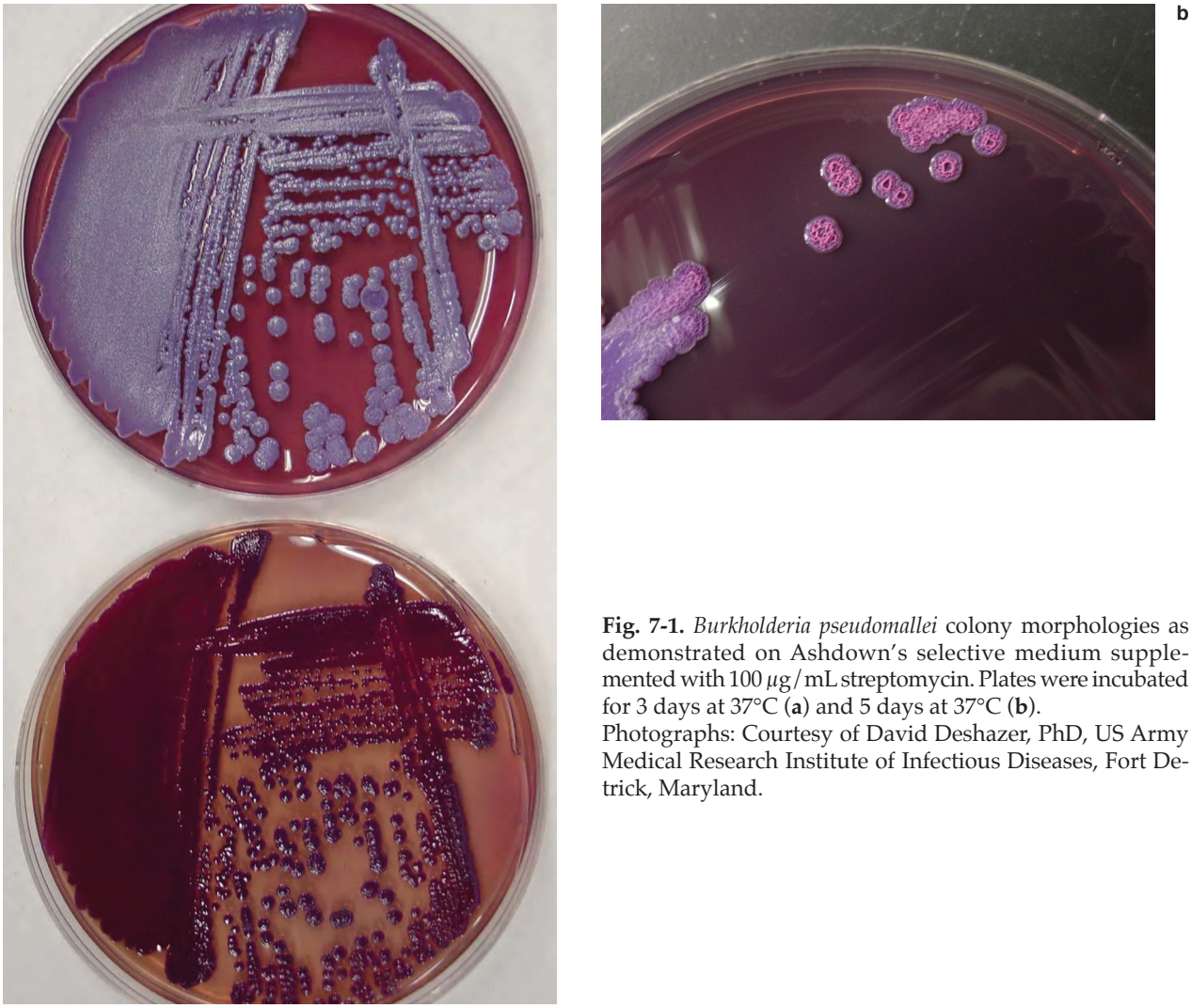

Fig. 7-1. *Burkholderia pseudomallei* colony morphologies as demonstrated on Ashdown's selective medium supplemented with 100 μg/mL streptomycin. Plates were incubated for 3 days at 37°C (**a**) and 5 days at 37°C (**b**).
Photographs: Courtesy of David Deshazer, PhD, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

metabolism).[51] 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 tRNA 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.[51] 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 caused by laterally acquired mobile genetic elements.[51-56] These genomic islands may provide strains that harbor a metabolic and/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.[27,57-59] Recently, the draft genome sequences of an additional nine *B pseudomallei* isolates (1710a, 1710b, 406e, 1106a, 1106b, S13, Pasteur 52237, 668, and 1655) were determined and deposited in Genbank, dramatically enhancing the amount and diversity of genome sequence data available for the study of *B pseudomallei*.

MILITARY RELEVANCE

Throughout the 20th century, melioidosis had an impact on the health of soldiers serving in Asia during times of war and peace.[60] Sporadic melioidosis infections occurred in US and Japanese soldiers during World War II,[38,61,62] and recrudescent melioidosis cases in World War II veterans were also reported.[63,64]

During the French Indochina War (1946–1954), there were at least 100 melioidosis cases among French forces during their fight against the resistance movement led by the Viet Minh.[19,60] Fewer than 300 melioidosis cases occurred among US soldiers during the Vietnam War,[19] and additional cases did not surface until years after the war's end, leading to the nickname "Vietnam Time Bomb."[65-67] Twenty-three melioidosis cases were reported in the Singapore armed forces from 1987 to 1994.[68] The infection rate in these relatively healthy servicemen was approximately 4-fold higher than the rate in Singapore's general population, 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 Category B biological terrorism agent that must be handled in biosafety level 3 laboratories.[9] Biosafety level 3 facilities incorporate specialized negative-air pressure ventilation systems, well-defined biosafety containment equipment, and protocols to study agents that can be transmitted through the air and cause potentially lethal infection. Category B agents have the potential for large-scale dissemination with resultant illness and death, but generally would be expected to have lower medical and public health impact than Category A agents.[9] *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.[69-71] However, *B mallei* was used as a biological warfare agent during the American Civil War, World War I, World War II, and in Afghanistan between 1982 and 1984.[2,70,72,73] The usefulness of *B pseudomallei* as a biological warfare agent is 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.

DISEASE

Epidemiology

Melioidosis cases have been increasingly 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.[11,13,20] Melioidosis has also been observed in the South Pacific, Africa, India, and the Middle East. In addition, sporadic melioidosis cases have occurred in the Western Hemisphere in Aruba, Brazil, Mexico, Panama, Ecuador, Haiti, Peru, and Guyana.[11,13,20] In endemic regions, the disease occurs in humans, sheep, goats, horses, swine, cattle, dogs, cats, and other animals.[15,24] Melioidosis cases that occur in temperate regions often result from recent travel to endemic areas.[18,74-77]

Pathogenesis

Several animal models of melioidosis have been developed to study pathogenesis, virulence factors, and efficacy of antibiotics and vaccines.[78-86] In general, hamsters and ferrets are highly susceptible to experimental melioidosis (median lethal dose [LD_{50}] of $< 10^2$ bacteria), and rats, pigs, and rhesus monkeys are relatively resistant (LD_{50} of $> 10^6$ bacteria). Infant rats can be made more susceptible to infection by intraperitoneal injection of streptozotocin, a compound that induces diabetes.[82,87] The LD_{50} of *B pseudomallei* for nondiabetic infant rats is greater than 10^8 bacteria in streptozotocin-induced diabetic infant rats; the LD_{50} is approximately 10^4 bacteria. Mice and guinea pigs exhibit intermediate susceptibility to experimental infection with *B pseudomallei*, but the LD_{50} for mice varies widely depending on the route of infection, mouse strain, and bacterial strain.[80,81,84,88]

Basic research on this pathogen has progressed rapidly over the past 5 years because of fears that *B pseudomallei* may be used as a biological weapon. The identification of virulence factors has been facilitated by the availability of genomic sequence data[51] and the existence of a nonpathogenic *B pseudomallei*-like species designated *B thailandensis*.[89-91] *B pseudomallei* and *B thailandensis* strains are genetically and immunologically similar to one another, but *B thailandensis* is avirulent in animal models of infection and rarely causes 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.[53,92,93] Exhibit 7-1 provides a brief description of all known *B pseudomallei* virulence factors, their mechanisms of action, and their relative importance in animal models of melioidosis.

B pseudomallei is a facultative intracellular pathogen that can replicate and survive in phagocytic and nonphagocytic cell lines.[94-99] After the initial phase of infection, researchers postulate that *B pseudomallei* can persist in a dormant stage in macrophages for months or years.[99] 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.

EXHIBIT 7–1

CANDIDATE VIRULENCE FACTORS OF *BURKHOLDERIA PSEUDOMALLEI*

Factor	Description
Capsule	A 200-kd group 3 capsular polysaccharide composed of a homopolymer of -3)-2-*O*-acetyl-6-deoxy-ß-D-*manno*-heptopyranose-(1-.[1] Capsule mutants are highly attenuated in hamsters and mice.[2,3] The capsule may contribute to survival in serum by reducing complement factor C3b deposition.[4]
TTSS	*B pseudomallei* harbors three distinct TTSS loci: (1) TTSS1, (2) TTSS2, and (3) TTSS3.[5] The TTSS1 and TTSS2 loci are similar to TTSS genes of the plant pathogen *Ralstonia solanacearum* and are not necessary for virulence in hamsters.[5] The TTSS3 locus is similar to the TTSS in *Salmonella* and *Shigella*[6] and is required for full virulence of *B pseudomallei* in both hamsters and mice.[5,7] The effector proteins of TTSS3 facilitate the invasion of epithelial cells and escape from endocytic vesicles.[6,8]
Quorum sensing	*B pseudomallei* encodes three *luxI* homologues that produce at least three quorum-sensing molecules: (1) N-octanoyl-homoserine lactone (C8-HSL),[9,10] (2) N-decanoyl-homoserine lactone (C10-HSL),[9,11] and (3) N-(3-hydroxyoctanoyl)-L-homoserine lactone (3-hydroxy-C8-HSL).[9] It also has five *luxR* homologues to sense these signals. Mutations in all of the *luxI* and *luxR* homologues result in strains with decreased virulence in hamsters and mice,[9,11] but the virulence-associated genes regulated by this complex quorum-sensing system are under investigation.
LPS O-antigen	An unbranched heteropolymer with repeating D-glucose and L-talose units with the structure -3)-ß-D-glucopyranose-(1–3)-6-deoxy-α-L-talopyranose-(1-.[12-14] LPS O-antigen mutants are attenuated in hamsters, guinea pigs, and infant diabetic rats and are killed by serum.[15] 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.[16]
Flagellin	A surface-associated 43-kd protein that is required for motility.[17,18] Flagellin mutants are attenuated in mice,[19] but not in hamsters or infant diabetic rats.[18] Passive exposure studies demonstrated that flagellin-specific antiserum was capable of protecting infant diabetic rats from challenge with *B pseudomallei*.[17]
Type II secretion	Required for the secretion of several exoproducts, including protease, lipase, and phospholipase C.[20] The products secreted by this pathway appear to play a minor role in *B pseudomallei* pathogenesis.[21]
Type IV pilin	*B pseudomallei* K96243 encodes four complete type IV pilin clusters.[22] A mutation in *pilA*, a gene encoding a type IVA pilin subunit, resulted 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.[23]
Biofilm formation	The extracellular slime matrix produced by *B pseudomallei* appears to be polysaccharide in nature, but the exact structure is unknown.[24] Biofilm mutants were not attenuated in the mouse model of melioidosis, suggesting that the biofilm plays a relatively minor role, if any, in virulence.[24]
Malleobactin	A water-soluble siderophore of the hydroxamate class.[25] The compound is capable of scavenging iron from both lactoferrin and transferrin in vitro.[26] The genes encoding malleobactin biosynthesis and transport were recently identified, but malleobactin mutants were not tested in animal models of melioidosis.[27]
Rhamnolipid	A 762-Da glycolipid with the structure 2-O-α -L-rhamnopyranosyl-α -L-rhamnopyranosyl-ß-hydroxytetradec anoyl-ß-hydroxytetradecanoate (Rha-Rha-C14-C14).[28] Rhamnolipid-treated cell lines exhibit profound morphological alterations, but the role of this glycolipid in virulence remains unknown.[29]
EPS	A linear unbranched polymer of repeating tetrasaccharide units composed of D-galactose and 3-deoxy-D-*manno*-octulosonicacid (KDO),with the following structure: -3)-2-*O*-Ac-ß-D-Gal*p*-(1-4)-α -D-Gal*p*-(1-3)-ß-D-Gal*p*-(1-5)-ß-D-KDO*p*-(2-.[30-32] 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.[31,33]
Endotoxin	The lipid A portion of *B pseudomallei* LPS contains amide-linked 3-hydroxyhexadecanoic acids,

(**Exhibit 7-1** *continues*)

Exhibit 7-1 *continued*

	which are longer than the fatty acid chains of enterobacterial LPS.[34] The endotoxic activity of *B pseudomallei* LPS was 10 to 100 times weaker than entobacterial 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.[34] The LD_{50} of purified *B pseudomallei* LPS in hamsters was 1,000 mg.[35]
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.[36-38] The autotransported protein BimA is located at the pole of the bacterial cell and is responsible for the formation of actin tails.[37] It is unknown if actin-based motility is required for virulence in animal models of melioidosis.
Exotoxins	There have been several reports in the literature about *B pseudomallei* exotoxins,[39-43] 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.[35,44] The K96243 genome sequence does not encode any homologues of known major toxins produced by other pathogenic bacteria.[22]

EPS: exopolysaccharide kd: kilodalton LPS: lipopolysaccharide TTSS: Type III secretion system

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(**Exhibit 7-1** *continues*)

Exhibit 7-1 *continued*

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Clinical Disease

Melioidosis is a tropical bacterial disease with primary endemic foci in Southeast Asia, northern Australia, south Asia, and China. Hyper-endemic areas for melioidosis include northern Australia and northeastern Thailand, where the disease incidence peaks in the rainy season. Heavy rainfall probably results in a shift from percutaneous inoculation to inhalation as the primary mode of infection, which leads to more severe illness.[100] In these hyper-endemic areas, *B pseudomallei* causes a substantial burden of infectious disease. For example, at a northeast Thai hospital that serves nearly 2 million rural rice-farming families, nearly 20% of all community-acquired bacteremia that occurred during the rainy season resulted from *B pseudomallei*.[101] Likewise, melioidosis is the most common cause of fatal community-acquired bacteremic pneumonia at the Royal Darwin Hospital in the Northern Territory of Australia.[102]

Cases of human-to-human transmission are rare, but have been documented.[103,104] The incubation period (time between exposure and appearance of clinical symptoms) is not clearly defined, but may range from 2 days to many years. Although serologic studies suggest that most infections with *B pseudomallei* are asymptomatic or mild,[105] individuals with risk factors, such as diabetes mellitus, alcoholism, cirrhosis, thalassanemia, or other immunosuppressed states, are at an increased risk of developing symptomatic infection. Other melioidosis-associated risk factors include chronic lung disease, excess kava consumption, and cystic fibrosis. Diabetes appears to be the most important of all the known risk factors because up to 50% of patients with melioidosis have diabetes mellitus.[24]

Melioidosis, which presents as a febrile illness, has an unusually broad range of clinical presentations that has resulted in various classifications of melioidosis, none of which are considered satisfactory.[106] However, clinical disease with *B pseudomallei* is generally caused by bacteria spread and seeding to various organs within the host. The diversity of infectious presentations includes acute localized suppurative soft tissue infections, acute pulmonary infections, acute fulminant septicemia, and chronic localized infections.[24] 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.[107,108]

Melioidosis is characterized by abscess formation. The majority of patients with melioidosis are septicemic. The lung is the most commonly involved organ—the nidus of infection is either a primary pneumonia or lung abscess, or the infection results from hematogenous seeding of the lung from bacteremia (Figure 7-2 and Figure 7-3). Patients with acute pulmonary melioidosis present with cough, fever, sputum production, and respiratory distress, and they 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

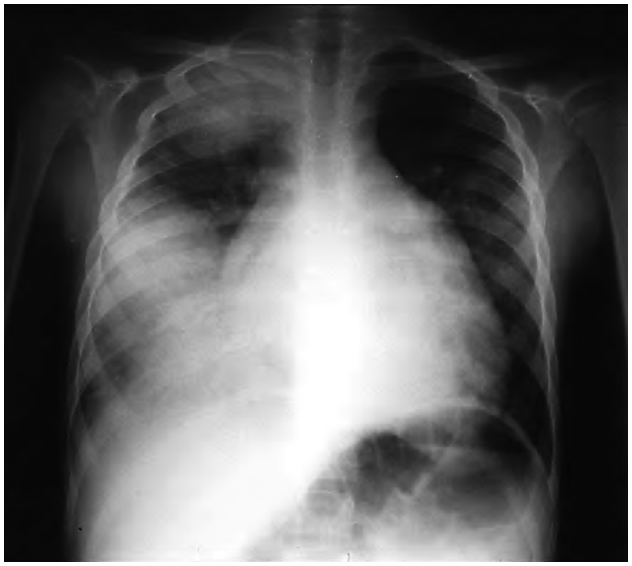

Fig. 7-2. Chest radiograph demonstrating a severe multilobar pneumonia.
Photograph: Courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

shock may appear on presentation. In an Australian study of 252 prospective melioidosis cases in the Northern Territory of Australia, 46% of the cases presented with bacteremia; in these cases the mortality rate was 19%.[102] Hematogenous seeding and abscess formation can occur in any organ (Figure 7-4). However, liver, spleen, skeletal muscle, prostate, and kidney are the most common abscess sites (Figures 7-5 and 7-6).[24]

Less common presentations of melioidosis include uncomplicated infections of the skin (Figure 7-7), subcutaneous tissues, or the eye. Corneal ulcerations resulting from trauma, which become secondarily infected with *B pseudomallei*, are rapidly destructive.[109] Septic arthritis and osteomyelitis (Figure 7-8) have also been described, but cellulitis appears to be rare. In a prospective study of more than 2,000 patients in Thailand, primary meningitis or endocarditis was not observed, but meningitis secondary to cerebral abscess rupture and mycotic aneurysms was seen.[24] Other unusual melioidosis presentations include mediastinal masses, pericardial fluid collections, and adrenal abscesses.

The clinical presentation of melioidosis varies among different regions. In Thailand 30% of the melioidosis cases in children present as acute suppurative parotitis.[110] These Thai 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.[24] Although acute suppurative parotitis is unusual

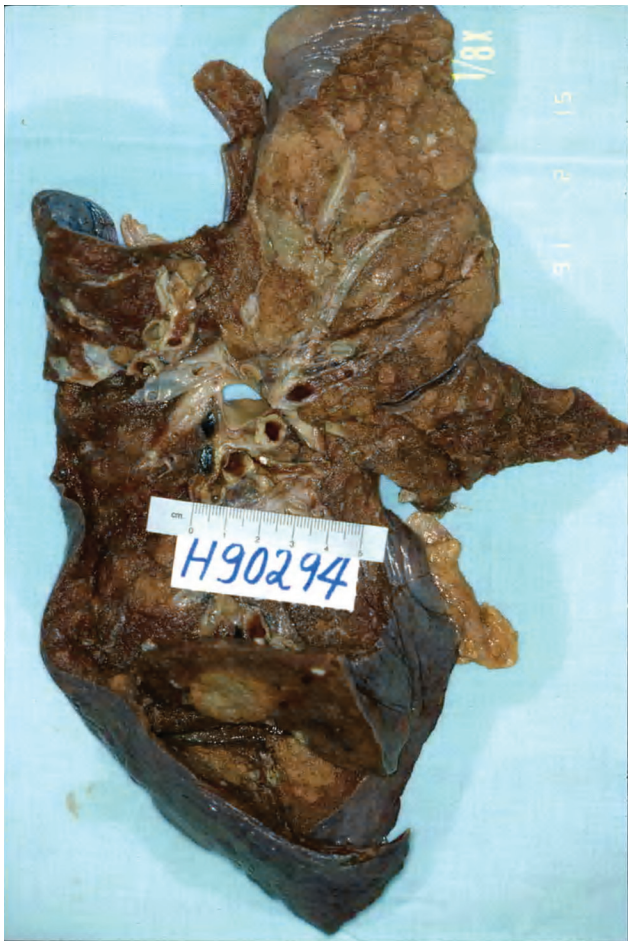

Fig. 7-3. Autopsy specimen demonstrating extensive pulmonary involvement with abscess formation resulting from *Burkholderia pseudomallei*.
Photograph: Courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

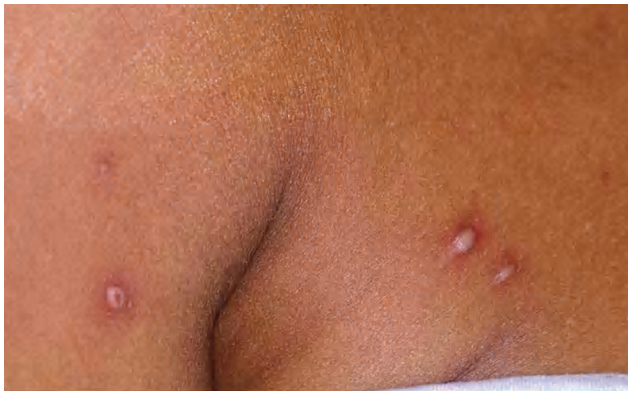

Fig. 7-4. Pustules with an erythematous base resulting from septicemic melioidosis.
Photograph: Courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

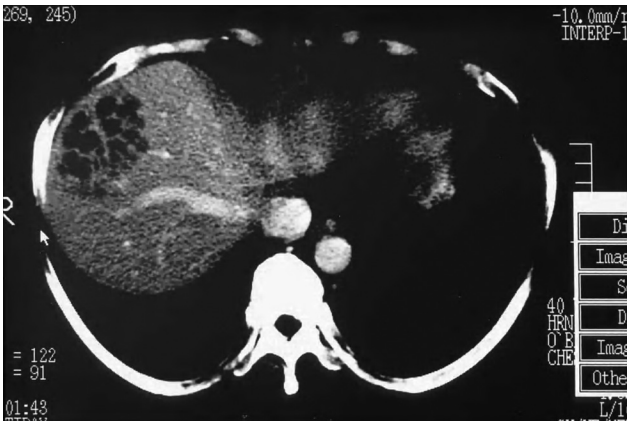

Fig. 7-5. Computed tomography scan showing multiloculated liver abscess.
Photograph: Courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

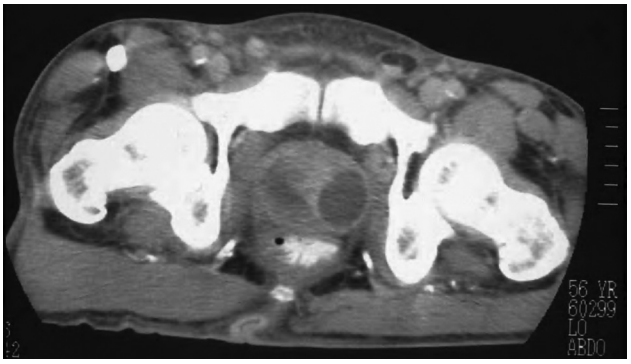

Fig. 7-6. Computed tomography scan showing prostatic abscess.
Photograph: Courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

in Australia, approximately 4% of the melioidosis cases in northern Australia present as brain stem encephalitis with peripheral motor weakness or flaccid paraparesis. Features of 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* microabscesses in the brain stem and spinal cord probably cause this syndrome.[24]

Although acute infections in individuals with predisposing risk factors are the most common, latent infection with reactivation, resulting in an illness that

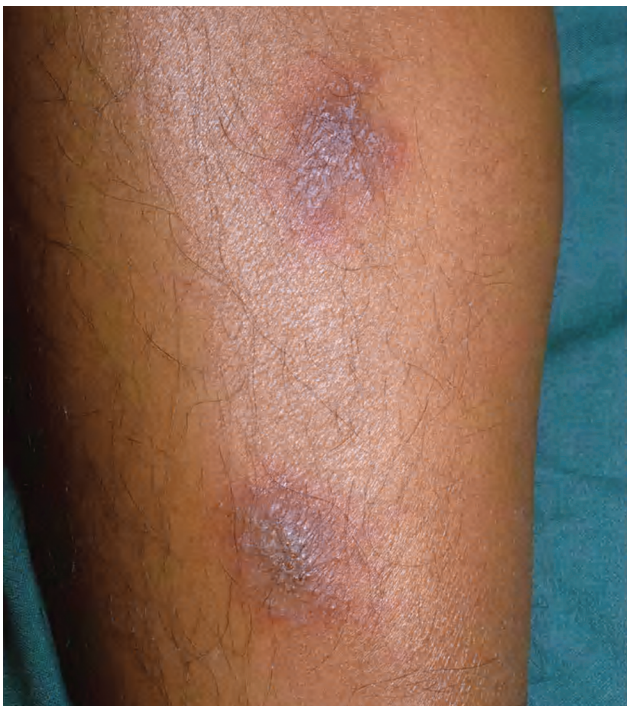

Fig. 7-7. Skin lesions associated with melioidosis on the lower extremity.
Photograph: Courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

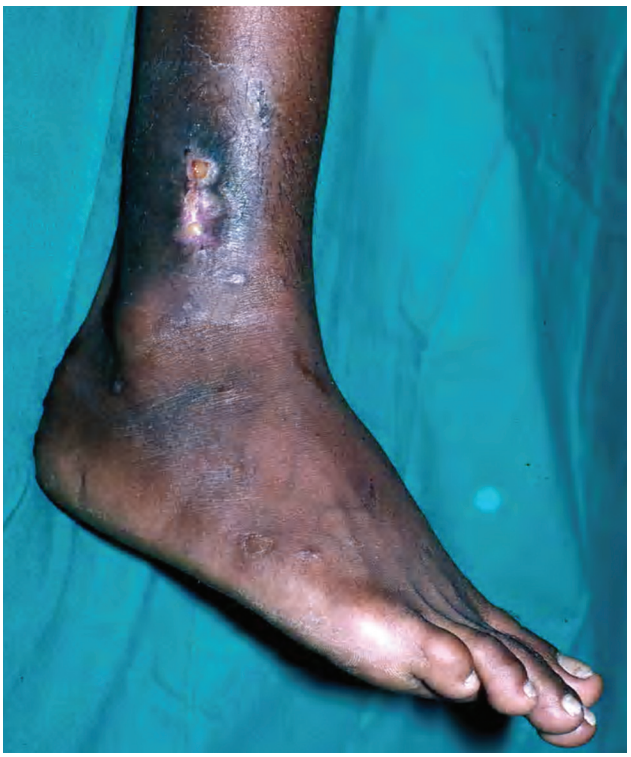

Fig. 7-8. Chronic osteomyelitis resulting from melioidosis.
Photograph: Courtesy of Bart Currie, MD, Royal Darwin Hospital, Australia.

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.[111] 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.[64] 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 because he had not returned to an area of melioidosis endemicity after being imprisoned in northwest Thailand, nor been exposed to individuals with melioidosis.[63] A recent study of recurrent melioidosis cases in northeast Thailand demonstrated that 75% were caused by the same strain (relapse) and 25% resulted from reinfection with a new strain.[112] Infection with *B pseudomallei* does not protect susceptible individuals from reinfection with a new strain.

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.[20] The organism is usually detected in blood culture within 48 hours. Ashdown's medium, a crystal violet and gentamicin-containing medium that permits selective growth of *B pseudomallei* (see Figure 7-1), has been used to significantly increase the frequency of recovery of *B pseudomallei* from the rectum, wounds, and sputum as compared with recovery on blood and MacConkey agars.[47] 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.[113,114] 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 lab. 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*,[46] although other investigators have reported mixed results.[115] The Vitek 1 (bioMérieux) has also been found to be highly sensitive, having identified 99% of the 103 *B pseudomallei* isolates tested.[116] However, in the same study, the Vitek 2 (bioMérieux) identified only 19% of these same isolates.[116] *B pseudomallei* identification was more reliable using the Vitek 2 colorimetric GN card when the correct software was used to analyze the data.[117]

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 and false positives, because of a high antibody prevalence to *B pseudomallei* in healthy individuals, in endemic areas.[108] 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.[118] Polymerase chain reaction-based identification techniques are also under development.[119,120]

Treatment

Asymptomatic carriage probably does not occur except for the apparent residual respiratory colonization in some patients with cystic fibrosis.[18] Therefore, the isolation of *B pseudomallei* from a clinical specimen requires treating the patient. All melioidosis cases—even mild disease—should be treated with initial intensive therapy (at least 2 weeks of intravenous [IV] therapy) followed by eradication therapy orally, for a minimum of 3 months. The choice of therapy for treating melioidosis is complicated because *B pseudomallei* is resistant to many antibiotics,[121,122] including aminoglycosides, first- and second-generation cephalosporins, rifamycins, and nonureidopenicillins. *B pseudomallei* is also relatively insensitive to quinolones and macrolides.[123] Therapeutic options are therefore limited.

The first study demonstrating the effectiveness of ceftazidime for severe melioidosis was published in 1989. In this study,[124] ceftazidime treatment (120 mg/kg/d) 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/d), doxycycline (4 mg/kg/d), trimethoprim (10 mg/kg/d), and sulphamethoxazole (50 mg/kg/d) (TMP-SMX). In 1992 a second randomized study for severe melioidosis conducted

in Thailand also demonstrated a substantial reduction in mortality ($P = 0.04$) when ceftazidime plus TMP-SMX was used, as compared to the four-drug conventional therapy.[105]

In 1999 a comparative treatment trial in Thailand found that imipenem was as effective as ceftazidime for treating 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.[125] Therefore, initial intensive therapy should be with high doses of ceftazidime (2 g IV every 6 hours, up to 8 g/d) or imipenem/cilastatin (1 g IV every 6 hours) or meropenem (1 g IV every 8 hours) combined with TMP-SMX (320 mg/1,600 mg IV or by mouth every 12 hours) for at least 14 days.[108] Critically ill patients with extensive pulmonary disease, organ abscesses, osteomyelitis, septic arthritis, or neurological melioidosis require longer intensive IV therapy.

The benefit of adding TMP-SMX to the initial antimicrobial regimen is supported by animal data and expert opinion.[23] However, a recent 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 TMP-SMX.[126] Nonetheless, all patients in the Northern Territory of Australia admitted to an intensive care unit for severe melioidosis are treated with meropenem and TMP-SMX. Meropenem is used rather than imipenem/cilastatin because it has fewer neurological side effects.[123]

The median time to resolution of fever is 9 days, but patients with large abscesses or empyema often have fluctuating fevers longer than 1 month. 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 medial therapy, prostatic abscesses usually require drainage to prevent treatment failures.[102] Adjunctive therapy with recombinant granulocyte colony-stimulation factor is routinely used for patients with melioidosis and septic shock in the Northern Territory of Australia. A retrospective review of mortality rates before and after the addition of granulocyte colony-stimulation factor therapy at the Royal Darwin Hospital was recently published. In this study, the introduction of granulocyte colony-stimulation factor as adjunctive therapy for patients with septic shock was associated with a decrease in mortality from 95% to 10%.[127] A randomized controlled trial to evaluate the efficacy of granulocyte colony-stimulation factor is under way in Thailand.[10]

After initial intensive therapy, oral maintenance therapy is given for another 12 to 20 weeks to prevent disease relapse. Oral maintenance therapy traditionally consists of chloramphenicol 40 mg/kg per day, doxycycline 4 mg/kg per day, and TMP-SMX 10 mg/50 mg/kg per day.[128] However, this combination frequently causes side effects resulting 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.[107] 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.[129] However, in the Northern Territory of Australia, TMP-SMX is used as monotherapy for maintenance therapy with a low relapse rate (1 failure in fewer than 60 patients).[102] Trials underway in Thailand are comparing the efficacy of TMP-SMX monotherapy with combination therapy.

Quinolones are not recommended for first-line therapy for eradicating *B pseudomallei*. Ciprofloxacin and ofloxacin were found inferior, with a failure rate of 29% (95% confidence interval 17%–43%) when compared to a 20-week course of maintenance therapy consisting of amoxicillin/clavulanate or the combination of chloramphenicol, doxycycline, and TMP/SMX.[130] Another study also found that the combination of ciprofloxacin plus azithromycin was associated with an unacceptably high rate of relapse.[131]

Prevention

Several experimental melioidosis vaccines have been tested in rodent models of infection, including live attenuated vaccines, heterologous vaccines, acellular vaccines, and subunit vaccines.[132] Variability in vaccination protocols, routes of challenge, and animal models makes 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.

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*.[133-137] *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.[138,139] 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.[138,140] 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*.[141] In contrast, vaccination with purified *bipD* protein did not significantly protect this animal model.[141] These studies suggest that live attenuated vaccines are promising candidates for melioidosis vaccines, but strains with defined deletion mutations should be examined 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 LD_{50} of wild-type *B pseudomallei*.[142] *B thailandensis* and *B pseudomallei* produce similar lipopolysaccharide (LPS) O-antigens and contain immunologically related secreted and cell-associated antigens,[89-90] which probably account for the protection that *B thailandensis* affords. The *B pseudomallei* exopolysaccharide and capsular polysaccharide (see Exhibit 7-1) are not produced by *B thailandensis*, and both polysaccharides may be necessary for full protection against challenge with *B pseudomallei*. Live attenuated *F tularensis* strains were also tested as heterologous vaccine candidates against melioidosis in rodents.[138,143] 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.[144] The vaccine consisted of a protein-polysaccharide mixture (1:3), and it significantly protected hamsters against experimental challenge with virulent *B pseudomallei*. In addition, the acellular vaccine reduced melioidosis mortality in cetaceans from 45% to less than 1%.[144] Unfortunately, the exact chemical components of the vaccine were not well characterized, leaving a high probability of lot-to-lot variation.

In a recent study, mice were actively vaccinated with purified *B pseudomallei* capsular polysaccharide or LPS and challenged with virulent *B pseudomallei* by the intraperitoneal or aerosol route.[145] 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 capsule had an increased mean time to death, but 100% of the vaccinated mice were dead by day 28.[145] 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.[81,100] Improved subunit vaccines that generate both humoral and cell-mediated immune responses are probably necessary to protect against infection with *B pseudomallei*.[146]

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.[18] Avoidance of *B pseudomallei* in the environment by those individuals with known risk factors is the only proven method of disease prevention. Animal studies have demonstrated the protective efficacy of doxycycline and to a lesser extent, ciprofloxacin, as prophylaxis against experimental melioidosis.[147] Based on these animal data, either doxycycline 100 mg by mouth twice daily or ciprofloxacin 500 mg by mouth twice daily may be recommended to individuals with risk factors and exposure to *B pseudomallei*. However, no clinical evidence suggests the efficacy of antibiotic prophylaxis in the prevention of human melioidosis.

SUMMARY

A disease caused by the gram-negative bacterium *B pseudomallei*, melioidosis 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 subtropical regions, and it is spread to humans through direct contact with the contaminated source and/or through inhalation. The majority of melioidosis cases have an identifiable risk factor, such as diabetes mellitus, alcoholism, cirrhosis, or other immunosuppressed states, although healthy people may develop disease. The incubation period is not clearly defined, but may range from 2 days to many years. Exposed individuals with a subclinical infection are potentially at risk for reactivation.

Melioidosis has an unusually broad range of clinical presentations. Disease is generally caused by bacteria spread and seeding to various organs within the host. Melioidosis is characterized by abscess formation. The majority of patients with melioidosis are septicemic. 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 isolate *B pseudomallei* from 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. Antibiotics recommended to treat melioidosis are ceftazidime, imipenem/cilastatin, or meropenem, each in combination with 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 postexposure prophylaxis in preventing human disease after exposure is unknown.

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