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Melioidosis

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Abstract

Burkholderia pseudomallei is a Gram negative environmental pathogen and the aetiological agent responsible for melioidosis, a fatal infection of humans (estimated ~89,000 deaths/year worldwide) and animals. Endemicity has been implicated across the tropics, but is most common in Southeast Asia and Northern Australia. Diabetes mellitus remains a major risk factor for acquiring melioidosis and poses a convergent threat alongside the global diabetes pandemic. Disease manifestations can range from acute fulminant septicaemia to chronic infection, whereby its facultative intracellular lifestyle and virulence factors promote survival and persistence within a broad range of cells. Clinical presentations and their severity may vary depending on the route of bacterial entry (inhalation, ingestion or skin inoculation), inoculation dose, bacterial strain and host immune function, but the majority of patients present with sepsis (a syndrome of life threatening organ dysfunction and dysregulated host response caused by an infection). Approximately 40-60% of patients are bacteraemic on presentation. With its large genome repertoire, *B. pseudomallei* can manipulate host immune responses and signalling to escape surveillance. Diagnosis is mainly based on clinical and epidemiological features as well as bacterial culture. Despite resurgence in research into this pathogen due to its biothreat potential, better diagnostic tests are needed to improve therapeutic efficacy and survival rates. Treatment requires prolonged intravenous and oral antibiotic courses to prevent relapse, and due to difficulties in laboratory diagnosis and clinical recognition, delays in treatment often lead to poor outcomes where mortality can exceed 40% in some regions even despite effective treatment.

[H1] Introduction

Melioidosis, "the great mimicker",[1] is an infectious disease caused by the environmental bacterium *Burkholderia pseudomallei*. First described over 100 years ago (Figure 1),[2] the organism is commonly found in the rhizosphere (layer of soil directly influenced by root secretions and soil microorganisms)[3] and surface water of many tropical and subtropical regions.[4,5] *B. pseudomallei* is an opportunistic, facultative intracellular, motile Gram-negative saprophyte (an organism that gets its energy from decaying organic matter), with an intrinsic armament of virulence factors and broad antimicrobial resistance that make it an imposing pathogen. *B. pseudomallei* is highly adaptable, allowing it to command a wide variety of clinical manifestations as well as survival advantage in infected hosts and the environment.[6] Naturally-acquired melioidosis in humans and animals results from inoculation, inhalation or ingestion of *B. pseudomallei*[7] with certain environmental conditions such as tropical storms, and occupation (e.g. rice farming) known to increase the risk of exposure.[8]

B. pseudomallei may result in an acute, chronic or latent infection, although most cases (85%) are considered to have acute infection from recent acquisition.[9] The majority of these patients will present with sepsis with or without pneumonia or localized abscesses (Figure 2). Subsequently, the diagnosis and management is often confounded by non-specific clinical signs and symptoms. The incubation period of acute infection is between 1 and 21 days (mean 9 days),[10] although more severe disease with shorter incubation may occur after inhalation or aspiration of fresh water.[11] The case fatality rate (CFR) of melioidosis is between 10-50% despite effective treatment.[8] For those who survive acute melioidosis, 5-28% of patients may experience recurrent infection, which may be due to relapse with the original strain or re-infection with a new strain following re-exposure.[8,12-14] Approximately 80% of patients have known risk factors, mainly diabetes.[15] The interplay between the host and pathogen is

complicated by its tropism for a wide variety of cells and ability to subvert and so avoid the host innate immune response.[16]

The closely related species *Burkholderia thailandensis,* which has a similar environmental niche but rarely if ever causes disease, is widely used as a surrogate for *B. pseudomallei* in laboratory studies of pathological mechanisms. *Burkholderia mallei*, a zoonotic pathogen and the aetiological agent of an infectious disease called 'glanders', is a host-adapted species originally derived from *B. pseudomallei* following substantial genome reduction. *B. mallei* is extremely infectious, mainly to solipeds but occasionally infects humans, and was used as a biological weapon in World War I.[8] The US Centers for Disease Control (CDC) have classified *B. pseudomallei* and *B. mallei* as tier 1 select agents because of their biothreat potential.[17] No vaccine for either is currently available,[18,19] further exacerbating concerns of an emerging public health threat.

This Primer summarises the state of the field in melioidosis research, focusing on epidemiology, pathophysiology including host-pathogen interactions, diagnostics, screening and prevention, and clinical management. Ending with an outlook, we will explore future directions of research in the 'omics' and cutting edge immunology era, argue whether melioidosis should be included in lists of neglected tropical diseases, and discuss whether a viable vaccine is on the horizon.

[H1] Epidemiology

[H2] Global burden and distribution

A modeling study in 2016 estimated that there are about 165,000 human melioidosis cases per year worldwide, of which 89,000 (54%) are estimated to be fatal (Figure 3).[4] This study implied that underdiagnosis and underreporting of melioidosis are a major issue, especially in the Indian sub-continent, where 44% of cases were predicted to occur, as there had been only about 1,300 human cases reported per year worldwide since 2010, less than 1% of the estimated annual incidence.[4]

The global importance of melioidosis is thus substantial, as the predicted mortality from melioidosis is comparable to that from measles (95,600 per year) and higher than for leptospirosis (50,000 per year) and dengue (12,500 per year), diseases that are considered to be of high priority by many international health organizations.[4] While melioidosis is well known to be prevalent in the Northern Territory of Australia and northeast Thailand, where the annual incidence is up to 50 cases per 100,000 persons,[5,9] the emergence of melioidosis in north-eastern Brazil is an example of increasing recognition of the disease in new areas due to enhanced awareness and improving diagnostics.[20] Although reports of *B. pseudomallei* isolation from soil and animals in equatorial Africa are limited, they suggest that melioidosis could be widely distributed across this region.[21-23] Nigeria is predicted to have one of the highest incidences of melioidosis in the world after India, Indonesia and Bangladesh.[4]

[H2] *B. pseudomallei* in the environment

B. pseudomallei is more readily detected in soil at depths of 10 cm or more than on the surface.[24] It has been postulated that *B. pseudomallei* can move from deeper soil layers during the rainy season as the water table rises to the surface, where it may then multiply.[24] A consensus guideline for soil sampling for *Burkholderia pseudomallei* was proposed in 2013,

with the goal of elucidating the global distribution of the organism.[24] However, culture of the organism in environmental samples is challenging, and molecular methods have greater sensitivity.[25]

B. pseudomallei can survive in extreme conditions, such as at least 16 years in water without nutrients (distilled water),[26] in nutrient-depleted soil[27] or in desert environments.[28] Outbreaks of melioidosis from contaminated water supplies have been reported from un-chlorinated water in the Northern Territory,[29] and associated with chlorination failure in Western Australia.[30] *B. pseudomallei* is also commonly found in un-chlorinated water supplies and drinking water in rural areas in Thailand.[31] Nosocomial infection has been attributed to *B. pseudomallei*-contaminated wound irrigation fluid, hand wash detergent, and chlorhexidine-cetrimide.[32,33]

Despite epidemiological evidence that melioidosis may be acquired by inhalation,[34] *B. pseudomallei* has rarely been detected in air. Aerosolised bacteria were first isolated in 1989 during a typhoon at the Hong Kong oceanarium.[35] Recently, *B. pseudomallei DNA* was detected from filtered air using qPCR in Taiwan following a typhoon[36] and the organism was isolated from air in Darwin (Northern Territory, Australia),[37] with whole genome sequencing linking the air isolate to the corresponding clinical isolate from a patient with mediastinal melioidosis.

[H2] Risk factors

Melioidosis can be found in all age groups, from the newborn to the elderly. Prospective studies in Australia and Thailand found the median age of patients with melioidosis was 50 years, with 5-10% of patients younger than 15 years of age.[9,38,39] Inoculation, ingestion and

inhalation of environmental *B. pseudomallei* are all important routes for the development of melioidosis.[7] Reported neonatal cases were probably due to mother-to-child transmission,[40] father-to-child transmission,[41] healthcare-associated infection[40] and community acquired infection.[40] One case has been associated with culture-positive breast milk.[42] Melioidosis is not contagious, and human-to-human transmission has rarely been reported.[8]

Risk factors predisposing to melioidosis are well recognised, most notably diabetes mellitus, which is present in more than half of all patients with melioidosis worldwide (box 1).[39,43] Two population studies have shown that diabetics have a 12 times higher risk of melioidosis after adjustment for age, gender and other risk factors.[39,43] Other known risk factors include exposure to soil or water, male gender (probably because of a greater risk of environmental exposure), older age, excess alcohol consumption and liver disease, chronic lung disease, chronic renal disease and thalassemia (likely causing neutrophil dysfunction due to iron overload).[8,44] Nonetheless, more than 80% of paediatric patients[38,45] and about 20% of adult patients have no recognized risk factors.[39,43] *B. pseudomallei* infections in adults who have no risk factors are exemplified by infections in soldiers, and particularly occur in those who have been exposed to a high bacterial inoculum, for example following aspiration of surface water.[46] Zoonotic transmission to humans is extremely rare, with only three possible zoonotic cases reported in Australia.[8]

[H1] Mechanisms/pathophysiology

Numerous studies and reviews have been published in recent years that have increased our insights into the pathogenesis of *B. pseudomallei* (Figure 4).[16,47-50]

[H2] The host defense against *B. pseudomallei* infection

[H3] Epithelial attachment and cell invasion

B. pseudomallei has a broad tropism for a variety of cell types, but inception of infection occurs at the epithelial cell interface via the mucosal surface or broken skin depending on route of entry. The mechanisms of cell invasion and replication are largely similar, and are, therefore, discussed collectively (unless otherwise specified).

The arsenal of known *B. pseudomallei* virulence factors is summarised in table 1 and box 2. *B. pseudomallei* possess multiple secretion systems (evolutionary apparatus that allows transport of proteins across cellular membranes so bacteria can respond to their environment, usually divided into different classes depending on their structure, function and specificity e.g. T3SS and T6SS), which on close contact with host cells trigger a molecular syringe (Structure made up of a filamentous needle to translocate effector proteins into surrounding milieu/cells).[51] The T3SS locus, *bsa*, contains genes that encode proteins for synthesis of both the secretion machinery and effector proteins.[52] T2SS is described as a terminal branch of the general secretory pathway (Gsp) and are widely distributed in Gram negative bacilli,[53] and auto-transporter proteins are secreted by T5SS, which are usually bound to the outer membrane with some acting like adhesins.

Attachment to human pharyngeal epithelial cells was initially thought to be mediated by capsular polysaccharide[54] and the type IV pili.[55] However, recent studies using acapsular mutants paradoxically found enhanced internalisation into human alveolar basal epithelial cells (A549) and HeLa cells.[56] Furthermore, a role for the type IV pilus gene, *pilA*, encoding a pilin subunit protein needed for adhesion to epithelial cells has been postulated.[57]

Flagella motility favour close contact with protective mucosal linings, but are unlikely to be a major adhesin for mammalian cells.[48,58] Two important proteins, BoaA and BoaB (Table 1), have been shown to enhance adherence. However, double knockouts show residual binding, indicating a requirement for multiple adhesins in eukaryotic cell adhesion.[59] BopE, which has activity as a guanine nucleotide exchange factor for Rho GTPases and a T3SS effector, causes rearrangement of the host actin cytoskeleton (membrane ruffling), facilitating ingress.[60] Mutation studies show that BidD, another effector, also exhibited impairment of invasion, which suggests that more than one T3SS effector mediates cell invasion.

Host cell factors also play a role in epithelial attachment. An example is protease-activated receptor-1 (subfamily of G protein-coupled receptors), which is expressed on several cell types (for example: endothelium, platelets and monocytes) and promotes cell incursion accompanied by *B. pseudomallei* growth and dissemination. Interestingly, inactivation had no effect on mortality in contrast to models with pneumococci which showed significantly delayed time to death.[61]

[H3] Intracellular survival

B. pseudomallei replicate intracellularly, cause lysis or spread to and infect adjacent/other cells. This causes acute symptoms, which may vary depending on tissue or organ infected.

B. pseudomallei can invade and propagate in both phagocytic and non-phagocytic cells.[62,63] Following phagocytosis, *B. pseudomallei* can be seen in vacuoles and later within the cytoplasm where they replicate.[64,65] Upon uptake, the vacuoles acidify rapidly with subsequent fusion with lysosomes.[66] The T3SS is one of the key mechanisms contributing to vacuolar escape, with multiple mutation models identifying downstream effects, including

reduced actin tail (polymerized host actin forms a comet-like filamentous tail used for intracellular motility) formation, intracellular survival, cytotoxicity and intracellular spread.[52,67-69] BsaQ (conserved inner membrane T3SS protein) mutants displayed 30% reduction in invasion.[48]

B. pseudomallei can multiply within phagocytes (including neutrophils, monocytes and macrophages) without activating a bactericidal response.[62,63] Despite detection of lysosome fusion within *B. pseudomallei* infected macrophages, surviving bacterial proliferation ultimately overwhelms the macrophage.[70] However, interferon (IFN)-γ-activated macrophages display enhanced killing of *B. pseudomallei*, probably via enhanced inducible nitric oxide synthase (iNOS) induction.[71]

Macrophage killing is predominantly due to reactive nitrogen intermediates (RNI) and reactive oxygen species (ROS).[71] Therefore, it is unsurprising that an important mechanism of *B. pseudomallei* pathogenesis is to suppress iNOS expression by upregulating two negative cytokines, a suppressor of cytokine signaling 3 (SOCS3) and cytokine inducible src homology 2 containing protein (CIS).[72,73]

Superoxide (O_2^-) and H_2O_2 degrading enzymes like SodC (superoxide dismutase), KatG (a catalase/peroxidase), AhpC (an alkyl hydroperoxide reductase) and DpsA (a DNA binding protein) have been associated with mediating *B. pseudomallei* oxidative stress resistance.[74-77] Additionally, AhpC assists the evasion of killing by RNI.[76] Intra-vacuolar survival is aided by an ecotin (a periplasmic serine protease inhibitor) homolog, which is involved in resisting the degradative enzymes within lysosomes.[78]

[H3] Evasion of autophagy and cell lysis

Autophagy is a well-regulated, cellular catabolic pathway by which unnecessary cellular components or bacteria are degraded and eliminated to maintain homeostasis.[79] *B. pseudomallei* triggers autophagy by a process stimulated by NOD2 activation[80,81] in a T3SS-dependent manner, resulting in bacterial killing.[82] However, the effectiveness of autophagy is influenced by T3SS BopA expression. Loss of BopA also leads to a significant delay in efficient phagosome escape, contributing towards virulence.[83] T3SS is likely to play a key role in evasion of autophagy, as demonstrated by increased uptake by autophagic vesicles of BopA mutants and decreased intracellular survival.[84] However, the complete mechanisms of autophagy are not comparable to other intracellular pathogens and escape remains to be defined further.

B. pseudomallei cytotoxicity for certain cell types is also strain-dependent. For example, some cause macrophage apoptosis,[65] some caspase-1 dependent cell lysis (pyroptosis)[68] and others have neither effect.[62] Macrophage lysis may represent an escape mechanism for *B. pseudomallei* once a threshold of bacterial replication has been reached.[50] By contrast, apoptosis and degradation of infected neutrophils by macrophages is delayed in melioidosis, favouring bacterial survival.[85]

[H3] Intracellular and secondary spread

As well as direct cell-to-cell or bactaermic spread, *B. pseudomallei* can also infect APCs, bringing them closer to the lymphatic system which may contribute to dissemination of infection.

Intracellular spread is facilitated by membranous protrusions that extend into neighbouring cells, through which bacteria travel by actin-mediated motility.[65,86] The autotransporter BimA interacts with monomeric actin where polymerisation occurs, forming 'comet tails' in the bacterial polar region.[87] Nerve root translocation of *B. pseudomallei* has been supported by animal studies[88] and linked especially to the minority $BimA_{Bm}$ genotype seen especially in Australia.[89] Such cell-cell spread along nerve roots is likely to explain the melioidosis encephalomyelitis syndrome, with brainstem disease following nasal/throat inoculation and myelitis potentially following skin inoculation on the limbs.[38] This results in cell fusion and the formation of multinuclear giant cells (MNGC),[90] a hallmark of melioidosis.[50] Although mechanistic studies are further needed, MNGC formation is also dependent on a functional T6SS-1 (synonymous with T6SS-5).[49] Eventual death of MNGCs results in plaque formation and subsequent damage to host cells, which may serve as a nidus for further *B. pseudomallei* replication or latent infection.[91]

B. pseudomallei can spread to secondary sites or blood causing sepsis. Currently, despite the exact mechanism of secondary spread remaining elusive, macrophage invasion allows a 'Trojan horse' transport process via lymphatics to disseminate to distant organs. Bacteria also remain viable in dendritic cells (DC), inducing maturation and trafficking to secondary lymphoid organs.[92]

[H3] Chronic and Latent infection

Chronic meliodosis is defined as symptomatic infection lasting longer than 2 months, occurring in 11% of cases in one 20 year prospective Australian study.[9] The host's immune response to acute infection consists of cytokine release (especially IFN-γ) and cell mediated defense, if successful, the infection may be controlled. An unknown percentage of people

exposed to *B. pseudomallei* could develop latent infection, and activation from latency has been estimated to account for <5% of overall melioidosis cases.[9]

Recurrent disease can be due to relapse or re-infection.[8,12-14] *B. pseudomallei* can remain latent for extended periods until activated by immunosuppression or other host stress responses. Reported latency periods until clinical disease have ranged from 19-29 years,[93-95] indicating that *B. pseudomallei* can enter a dormant state and evade immune surveillance.[50] Neither the site (tissue or subcellular level) of latency nor mechanisms by which *B. pseudomallei* remains undetected are clear,[96] although years later following an episode of acute melioidosis high antibody titres in patients may suggest continuous exposure or covert sequestration.[97] *B. pseudomallei* has also been recently found within the nuclear compartment, potentially acting as a sanctuary site for later recrudescence of infection.[98] Strain variability or small colony variants (SCV) may also play a role in determining whether persistent infection is established.[99]

Some *B. pseudomallei* persistence factors have been characterized, including toxin-antitoxin (TA) systems, metabolic enzymes and adaptive mutations.[100] By entering a slower growth rate, TA systems allow bacteria to survive under stressful environments, while SCV can shift to an acid-tolerant state such as found in abscesses.[101,102] The resistance patterns seen can obviously be selected for by host and antibiotic pressure. For example, variation in TetR family repressor showing negative selection for function and near genes such as multidrug efflux pumps, penicillin-binding proteins and β-lactamases may occur (see Box 3).[103] The studies described showed multiple genotypes within a single infection, at least partly resulting from genetic adaptation to the human host, including inactivation of virulence and immunogenic factors, as well as deletion of pathways involved in environmental survival.

Thus, bacterial isolates from individual patients with persistent or recurrent infection show extensive adaptive regulatory changes, favouring bacterial persistence. This included genome reduction and increased antibiotic resistance. Data do not yet support a significant correlation between phages and pathogenicity in the *B. pseudomallei*.[103,104]

[H2] Host-pathogen response

Most patients with melioidosis have one or more predisposing risk factors, suggesting that initiation, progression and outcome of disease are largely determined by host immune status.[15,96] For example, genetic polymorphisms in tumour necrosis factor (TNF)-α gene allele 2, nucleotide-binding oligomerization domain-containing protein (NOD)-2 region, Toll-like receptor (TLR)-4 and TLR5 have all been linked to severity of disease in melioidosis patients.[80,105-107] Hypofunctional TLR5 was associated with decreased organ failure, improved survival and functional cytokine response, possibly mediated by interleukin IL-10.[108] Interestingly, human carriers of hyporesponsive TLR5 polymorphism display heightened susceptibility to invasive aspergillosis and Legionnaire's disease.[109]

[H3] Innate immune response

B. pseudomallei activates the alternative complement pathway, but bactericidal activity of the membrane attack complex is obstructed as it attaches to the external capsule polysaccharide.[110] Due to its capsule and lipopolysaccharide (LPS), *B. pseudomallei* is also resistant to lysosomal defensins and cationic peptides, allowing survival in human serum and within phagocytes.[50]

Acute melioidosis results from an ineffective cellular immune response. Neutrophil, macrophage and lymphocyte recruitment at the point of infection are triggered by the immune response initiated by pattern recognition receptors. Despite the possible detrimental effects of excessive neutrophil recruitment,[111] activated neutrophils play a pivotal role in early bacterial containment.[112]

TLRs recognise conserved pathogen-associated molecular patterns (PAMPs) and mediate an inflammatory immune response via various signaling adaptor proteins, including myeloid differentiation factor 88 (MyD88) and TIR-domain-containing adaptor-inducing interferon-β (TRIF) (Figure 4). The importance of the TLR signaling pathway is underscored by the notion that MyD88 downregulation in experimental melioidosis increases susceptibility as a result of diminished neutrophil recruitment and activation.[113] *B. pseudomallei* triggers TLR2, TLR4 (and its co-receptor CD14) and TLR5 receptors in host cells, as well as inducing IL-8 production via NF-κB.[114-116] It should be noted that structural diversity of *B. pseudomallei* LPS affects innate immune signaling. In addition, its recognition seems to be model dependent, occurring solely through murine TLR4, while in human models TLR2 plays an additional role.[117]

TLR signaling may be dampened or dysregulated by LPS,[118,119] and increased expression of SOCS3, CIS, SARM-1 or SIRPα may contribute to failed TRIF-dependent TLR pathway activation during melioidosis. Loss of phagocytic killing capacity may also be attenuated by SOCS3 and CIS through inhibition of JAK/STAT signaling and macrophage response to interferons.[72] Non-survivors of septic melioidosis also show increased levels of mononuclear cell expression of interleukin 1 receptor associated kinase (IRAK) like molecule (IRAK-M) and NF-κB,[73,120] perhaps signifying the dysregulated host response which leads to organ dysfunction in sepsis.

Phagosomal escape exposes *B. pseudomallei* to intracellular TLR-independent PRPs, namely NOD-like receptors and by extension to the apparatus of the inflammasome.[121] Sensing of *B. pseudomallei* PAMPs, results in activation of caspase-1 in early infection, followed rapidly by pyroptosis of infected cells.[122] (Figure 4) Additionally, NLRP-3 activation of caspase-1 releases active interleukin-1ß (IL-1ß) and IL18 which are both increased in patients with septic melioidosis.[111,120,123,124] IL-18 protects against *B. pseudomallei* infection due to IFN-γ induction properties,[111,123] while IL-1ß plays a deleterious role due to excessive recruitment of neutrophils. This interplay supports intracellular growth of *B. pseudomallei*, tissue damage, and inhibition of IFN-γ production.[111]

[H3] The inflammatory response

Acute melioidosis leads to a dysregulated cytokine-mediated immune response resulting in excessive inflammation.[96] Elevated levels of pro-inflammatory cytokines (IL-6, IL-12, IL-15, IL-18, TNF-α and IFN-γ) are observed, some of which have been correlated with a fatal outcome (e.g. IL-6 or IL-18, considered mortality predictors).[123,125] *B. pseudomallei*-stimulated IFN-γ production activates T-cells and natural killer (NK) cells, sustaining a cell mediated immune response. NK cell-derived IFN-γ is paramount for fighting infection, as demonstrated in knockout murine models and by virtue of the fact that NK cells are detected at the site of infection, producing 60-80% of the secreted IFN-γ.[112,126]

B. pseudomallei interaction with the macrophage cell surface leads to predominant expression of TNF-α. Abrogation of TNF-α or its receptors (in knockout models) results in susceptibility to melioidosis, with increased neutrophil-based inflammatory influx and associated necrosis.[112,127,128]

Anti-inflammatory cytokines (IL-10, IL-4, TNFR1 and IL-1RA) are also upregulated during septic melioidosis. Significant increases in IL-1RA and TNFR1 expression are seen in non-survivors.[120,129] Late onset inflammatory mediators, such as high mobility group box 1 protein (HMGB1) and macrophage migration inhibitory factor (MIF)[130] expression, are also correlated with clinical outcome and mortality.[131]

[H3] Adaptive immune response

Despite *B. pseudomallei* antibodies being common in persons from melioidosis-endemic regions (either due to past or asymptomatic infection), their role in developing functional immunity to melioidosis is ambiguous, with re-infection possible from different strains. Rather, recurrent infection can occur even in the presence of high antibody levels.[97].[132,133]

Upon intracellular invasion by *B. pseudomallei*, a strong comprehensive cell-mediated immune response, with T-cells playing an important role, is essential for protection against progression of infection and bacterial clearance.[134] CD4 T-cells are paramount for B-cell isotype switching, activation of CD8 cells and triggering phagocytes.[135] Consistent with this, human survivors display an increased frequency of CD4+ and CD8+ T-cells, while a decrease is specifically correlated with greater mortality. Moreover, vaccines evoking a Th1-skewed immune response provide protection against melioidosis, with potential to generate sterilizing immunity.[136,137]

Gradually, granulomas form at the site of infection, which contain neutrophils, macrophages, lymphocytes and giant cells. Intracellular 'globi' of bacilli with giant cells, sometimes resembling Langerhan's giant cells are seen in a background of acute necrotising inflammation.[138]

In persistent infections, murine models show reduced CD4+ T-cells and upregulation of CD8+ T-cells, with both having increased expression of PD-1, but unchanged expression of cytotoxic T lymphocyte associated protein 4 (CTLA-4). Interestingly, heightened expression of PD-1 may be linked to T-cell exhaustion in chronic melioidosis,[139] as well as inhibiting proliferation and cytokine production.[140]

Surprisingly, HIV infection does not appear to be a risk factor for acquiring melioidosis or for more severe disease/fatal outcome.[141] This is at odds with other organisms with similar pathogenicity mechanisms like non-typhoidal *Salmonella* (NTS).[142] Previously it has been demonstrated that macrophages in HIV individuals show a dysregulated cytokine response (TNF-α, IL-10, IL-12), related to CD4 cell count, but retaining bacterial internalisation and intracellular killing.[143] Depletion studies of T-cells and NK cells (hence reduction in 95% of the IFN-γ production) did not hamper bacterial control, suggesting substantial redundancy, minimal level for clearance of bacteria and importance of MHC-II macrophages.[144] For primary melioidosis it has been suggested that bystander T-cell activation is not required for host survival and may play a more significant role during the antigen-induced phase.[144] Paradoxically, recent findings found a strong CD4+ and CD8+ response during acute infection, with an association between lower responses and mortality from disease.[136]

[H1] Diagnosis, screening and prevention

Melioidosis is grossly under-diagnosed worldwide.[4] The main reasons for this are a lack of diagnostic microbiological laboratories serving the poor rural populations who are at greatest risk of infection, and a lack of awareness of the disease amongst physicians and laboratory

482 staff. Even good microbiological laboratories may initially miss the diagnosis and discard *B.*
483 *pseudomallei* as a contaminant, especially in non-endemic areas.[145]
484
485 **[H2] Clinical Diagnosis**
486 Inoculation with *B. pseudomallei* usually results in subclinical disease, with the majority of
487 immunocompetent individuals clearing the infection. This is supported by the fact that there
488 were only 343 reported melioidosis cases amongst 225,000 American soldiers estimated by
489 serological studies to have had exposure to *B. pseudomallei* in Vietnam.[146] An unknown
490 proportion of those infected may have persisting latent infection. In those who develop
491 clinical illness (i.e. melioidosis), the clinical presentation, severity of illness, and outcome
492 are all influenced by the presence or absence of risk factors, the route of infection, the
493 infecting bacterial load and, importantly but not predominantly, the presence or absence of
494 specific non-ubiquitous *B. pseudomallei* virulence genes.[15,89] The clinical spectrum of disease
495 ranges from localized cutaneous infection at an inoculation site with no systemic
496 manifestations to overwhelming sepsis and death (Figure 5). Bacteremia on admission occurs
497 in 40 to 60%, with septic shock in around 20% of all cases. Pneumonia is the presenting illness
498 in about half of all cases. Dissemination to internal organs is common, especially the spleen,
499 prostate, liver and kidneys. Making a diagnosis on clinical grounds alone is thus very
500 difficult, although in known endemic areas a patient with suggestive clinical and
501 epidemiological features (e.g. a diabetic rice farmer who presents during the rainy season
502 with sepsis, pneumonia and liver and splenic abscesses) should be treated empirically with
503 antibiotics that cover melioidosis.
504
505 **[H2] Microbiological Diagnosis**

Aspects of laboratory diagnosis of melioidosis, including common misconceptions and pitfalls, were reviewed recently.[147] Key points are discussed below.

[H3] Culture

Culture remains the mainstay of melioidosis diagnosis. *B. pseudomallei* can grow on most routine laboratory media, but may not be recognised unless laboratory staff are familiar with its appearance, and it may be labelled as '*Pseudomonas* species', 'non-fermenter', *Bacillus* species or other environmental organism, and dismissed as a contaminant.[147] In fact, *B. pseudomallei* is never found as part of the normal human flora, and so its isolation from any clinical sample should be regarded as diagnostic of melioidosis. Given its status as a Hazard Group 3 pathogen and Tier-1 Select Agent[148], doctors should alert the laboratory if patients are suspected of melioidosis so that locally appropriate precautions can be followed. All microbiology laboratory staff in melioidosis endemic areas should receive appropriate training regularly and follow local safety standards.

While blood cultures are positive in over 50% of patients overall,[9] the proportion is lower in children,[45] potentially reflecting their usual good health and lack of classical melioidosis risk factors. Positive blood cultures have been reported as high as 75% in some recent series.[149,150] Other samples that should be cultured in patients suspected of having melioidosis include pus from abscesses, sputum in patients with pneumonia, and urine in patients with urinary symptoms. Culture of throat (or rectal) swabs[151,152] and the centrifuged deposit of urine[153] using selective media such as Ashdown agar and broth[154] is also worthwhile in all cases of suspected melioidosis, as these may be the only positive samples in some patients. Nonetheless, sampling should not delay timely parenteral antimicrobials. Due to low sensitivity (60%),[155] in patients who are strongly suspected of having melioidosis, repeating

cultures (especially blood, sputum, urine and pus) may be worthwhile, as it is not uncommon to find patients in whom subsequent samples are culture positive despite initially negative results. On the other hand, if after 3 to 7 days patients do not improve despite appropriate treatment for melioidosis and all culture results are negative, re-thinking of the cause, further investigations, and a change of treatment should be considered.

B. pseudomallei grows on most routine laboratory media, but relatively slowly compared with many other organisms so it may be outgrown in samples from sites with a normal flora, hence the value of selective media. Agar plates should be inspected daily for 4 days if patients are suspected of melioidosis. The difficulty is recognising *B. pseudomallei* colonies unless laboratory staff are familiar with this. Standard biochemical tests and kit-based identification methods, such as API20NE and VITEK (bioMerieux, Crappone, France), can be used to confirm the identity, although misidentifications of *B. pseudomallei* as other bacteria with those methods are not uncommon.[147] A serological approach using monoclonal antibody-based latex agglutination is very useful for screening suspect colonies.[156] Increasing numbers of clinical laboratories are using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) for bacterial identification, and this provides an accurate identification of *B. pseudomallei* as long as the organism is represented in the reference database.[157] A large number of molecular approaches for species identification have been described, including 16s rDNA sequencing[158] and specific PCRs,[159] although these are often only available in research and reference laboratories. The antimicrobial susceptibility pattern of *B. pseudomallei* is very characteristic and in resource-limited areas, a simple 3 disc diffusion test (resistance to gentamicin and colistin/polymyxin with susceptibility to co-amoxiclav) has been recommended for screening oxidase-positive Gram negative rods,[147,160]

555 although gentamicin-susceptible isolates predominate in some regions.[161] Assays are being
556 developed to detect the known *B. pseudomallei* resistance genotypes.
557
558 **[H3] Direct Detection in Clinical Samples**
559 Given the seriousness of melioidosis, the delays introduced by waiting for bacteria to grow
560 could have fatal consequences, and efforts have been made to speed up the diagnosis by
561 direct detection of the organism in clinical samples. Although the organism is often described
562 in text books as a Gram negative rod exhibiting 'safety pin bipolarity', light microscopy lacks
563 sensitivity and specificity.[162] Better results can be achieved using immunofluorescent
564 microscopy, with a specificity approaching 100%, although the sensitivity is less than 50%
565 compared with culture.[163]
566
567 Alternative approaches using antigen detection or nucleic acid amplification have also been
568 employed. A simple lateral flow immunoassay targeting the extracellular polysaccharide has
569 been developed,[164] although it has not yet been extensively evaluated and despite good
570 specificity it appears to lack sensitivity, especially for blood.[165] Numerous PCR assays with
571 high specificity for *B. pseudomallei* have been developed over the past 20 years and have
572 undergone relatively small-scale clinical evaluations. The most promising assay targets the
573 type III secretion system gene cluster,[166] although there are also problems with sensitivity
574 when testing blood.[167] However, as yet none of these PCR assays has found a place in routine
575 clinical diagnosis in endemic areas, even in high-income countries such as Singapore and
576 Australia. This is largely because the current PCR assays lack sensitivity and are not cost-
577 effective in providing the information required by clinicians in order to make therapeutic
578 decisions.
579

[H3] Serology

There are numerous problems with the serodiagnosis of melioidosis. Many different assays have been developed for detecting antibodies to *B. pseudomallei*, but most use poorly characterised antigens and have never been internationally standardised or subjected to extensive critical evaluation. The most widely used is an indirect haemagglutination (IHA) test. The background seropositivity rates in some endemic areas are very high resulting in low specificity, presumably because of the repeated exposure to *B. pseudomallei* or closely related organisms in the environment.[168,169] As a result, many non-melioidosis patients presenting with fever are currently misdiagnosed as having melioidosis on the basis of the IHA positivity in endemic countries in Southeast Asia. Conversely, some patients with melioidosis never mount a good antibody response, perhaps reflecting underlying immunocompromise. The IHA test on admission has a reported sensitivity of only 56% in Australia[170] and 73% in Thailand,[169] although 68% of the negatives in the Australian study subsequently seroconverted.[170] As a result, the IHA should not be relied on as a diagnostic test for melioidosis.

More recently a range of novel assays using purified antigens as targets are being developed and have undergone small-scale evaluations, with some evidence of improved sensitivity and specificity.[171-173] A recent example is a protein microarray, which contains 20 recombinant and purified *B. pseudomallei* proteins that provides a standardized, easy-to-perform test for the detection of *B. pseudomallei*-specific antibody patterns.[174] Such a system could have the potential to improve the serodiagnosis of melioidosis in clinical settings.

[H2] Screening

The key to diagnosing melioidosis is ensuring that the right samples (see above) from the right patients are sent for culture to laboratories familiar with the disease and its causative organism.

Since the clinical presentation of melioidosis can be non-specific, it is important to consider the diagnosis in anyone with a fever in endemic and potentially endemic countries (list of the countries available in the modeling study[4]). This is even more important in those with abscesses, especially in the liver, spleen, prostate or parotid, or pneumonia. Other suggestive features include a history of contact with soil or surface water, and any of the associated underlying conditions, particularly diabetes mellitus, chronic kidney, liver or lung disease, haematological problems such as thalassaemia or leukaemia, and long-term steroid use. However, some patients will have none of these risk factors, and so melioidosis should be part of the differential diagnosis for anyone with a fever in a known endemic area, particularly if they fail to respond to the more usual treatments given to patients with suspected sepsis or pneumonia such as ceftriaxone, aminoglycosides, macrolides, or anti-tuberculous drugs.

[H2] Prevention

Considerable efforts are made each year in northern Australia to give basic public health advice, especially to high risk groups, about avoiding direct contact with soil and water at the start of each rainy season.[175] In Thailand, evidence-based guidelines for the prevention of melioidosis recommend that residents, rice farmers, and visitors should wear protective gear such as boots and gloves if direct contact with soil or water is necessary, should only drink bottled or boiled water, and should avoid outdoor exposure to heavy rain or dust clouds.[7] The guidelines also encourage cessation of smoking (particularly in those with underlying

conditions such as diabetes), and discourage the application of herbal remedies or organic substances to wounds.[7] However, the effectiveness of this advice in reducing the incidence of infection has not been proven. Outbreaks of infection have been associated with contaminated potable water supplies, especially when water treatments have failed.[176] Ideally individuals at risk should not consume untreated water, and should wash food to be eaten raw using boiled or bottled water.[7] Chlorination is only partially effective.[177] In low and middle-income countries, water should be boiled before consumption. UV treatment is effective for remediation of water contaminated with *B. pseudomallei*, and may be recommended in high-income countries in households where individuals are at heightened risk of contracting melioidosis.[178]

There are numerous barriers to the adoption of behaviours recommended for melioidosis prevention in developing tropical countries.[179] Public awareness of melioidosis is highly limited.[179] Over-the-knee boots, hip boots and half-body waders can be used in flooded rice fields without causing difficulty in walking, but may still be uncomfortable in hot weather. A multifaceted intervention at community and government level would be required for the prevention to be successful, and such an intervention is currently being prospectively evaluated in north-east Thailand.

If high-risk laboratory exposure to *B. pseudomallei* occurs, oral antimicrobial treatment with trimethoprim-sulfamethoxazole or doxycycline or amoxicillin-clavulanate (if the organism is resistant to trimethoprim-sulfamethoxazole or patient is intolerant) for 21 days is recommended.[180] The potential benefit of post-exposure prophylaxis must be weighed against the fact that trimethoprim-sulfamethoxazole can have severe adverse effects. High-risk incidents include penetrating injuries contaminated with *B. pseudomallei*, splashes leading to

contamination of the mouth or eyes, and generation of aerosols outside of a biological safety cabinet.[180] For persons involved in a low-risk incident, the decision to begin post-exposure prophylaxis (PEP) should be based on the presence of known risk factors for natural melioidosis. Persons with known risk factors should be advised PEP, whereas persons with no known risk factors should be managed with monitoring alone.[180] Confusion about when PEP should be used is not uncommon.[181]

[H1] Management

The two critical aspects for therapy of melioidosis are early diagnosis and commencement of specific antimicrobial therapy. In locations with resources for rapid diagnosis, early implementation of optimal antibiotic therapy and state-of-the-art intensive care facilities for managing severe sepsis, mortality is around 10%.[15] Nevertheless, such resources are not available or are limited in many of regions where melioidosis is endemic, and in those circumstances mortality remains at 40% or higher.[15] From the late 1980s, ceftazidime became the drug of choice for initial intensive therapy for melioidosis[182]. The vast majority of *B. pseudomallei* isolates from primary melioidosis infections have the same characteristic antimicrobial susceptibility profiles, being susceptible to ß-lactam antibiotics such as ceftazidime, imipenem, meropenem and amoxicillin-clavulanate, but with considerable degrees of variation in bactericidal activity. Most are also susceptible *in vitro* to piperacillin, ceftriaxone and cefotaxime, although the latter agents appear less effective clinically.[183] Recent studies have confirmed that primary isolates are also almost always susceptible to doxycycline, chloramphenicol and trimethoprim-sulfamethoxazole.[184-186] Although these agents have only bacteriostatic activity, there are historical reports of the successful use of trimethoprim-sulfamethoxazole alone and tetracycline or doxycycline alone for melioidosis.

B. pseudomallei is resistant to penicillin, ampicillin, first- and second-generation cephalosporins, gentamicin, tobramycin, streptomycin, macrolides, quinolones and polymyxins (box 3). An exception is the occasional gentamicin susceptible isolate, with a clonal group of these recently found to be common in Sarawak, Malaysia.[161]

New antimicrobials have been tested *in vivo* and in animal models, but none to date is likely to replace the current primary role of ceftazidime and meropenem.[187] Doripenem has minimum inhibitory concentrations (MICs) similar to meropenem but ertapenem, tigecycline and moxifloxacin appear to have limited *in vitro* activity.[188]

The current guidelines for melioidosis therapy have evolved from sequential randomized comparative antibiotic trials from northeast Thailand, commencing in 1986, as summarised in a recent review.[187] Formal guidelines, including recommended dosage and duration of each therapeutic phase, have been published by the CDC following a 2010 expert workshop which updated prior consensus guidelines.[180] Antimicrobial therapy is separated into the initial intensive phase and the subsequent eradication phase (table 2).

As melioidosis is not a contagious disease, melioidosis patients are not usually managed in isolation or with special precautions within endemic areas. Healthcare providers are recommended to follow universal precautions[189] and standard infection control including hand hygiene.[190] However, nosocomial infections have been reported[32,191,192] and there is at least a theoretical risk for contamination of the ward environment from patients with superficial lesions or pneumonia, so it would be wise to nurse patients in standard isolation in non-endemic areas.

[H2] Initial intensive therapy

Intravenous ceftazidime or meropenem is the preferred choice. The duration of initial intensive therapy should be a minimum of 10 to 14 days, with longer intensive therapy for critically ill patients, extensive pulmonary disease, deep-seated collections or organ abscesses, osteomyelitis, septic arthritis, and neurologic melioidosis (table 2). Therapeutic response can be slow; median time to resolution of fever is up to 9 days,[193] with longer times in those with deep-seated abscesses. The addition of trimethoprim-sulfamethoxazole to ceftazidime for the intensive phase conferred no survival benefit in Thai studies.[194,195] Nevertheless because trimethoprim-sulfamethoxazole has excellent tissue penetration, some authorities recommend combination therapy specifically for neurologic, cutaneous, bone, joint and prostatic melioidosis.[15,180]

Imipenem and meropenem have the lowest MICs against *B. pseudomallei*, with *in vitro* time-kill studies measuring the rate of bacterial killing showing that these carbapenems perform better *in vitro* than ceftazidime against *B. pseudomallei*.[196,197] The recommendation of meropenem as the drug of choice for severe melioidosis with septic shock is also supported by observational data from Australia suggesting that meropenem confers better outcomes in severe melioidosis than ceftazidime.[198] However it remains important to note that ceftazidime remains the drug of choice for initial therapy for most patients with melioidosis, with no evidence that meropenem is superior to ceftazidime in those who are not critically ill.

[H2] Subsequent eradication therapy

After initial intensive therapy, follow-on eradication therapy is recommended to prevent recrudescence or later relapse of melioidosis. The most recent trial showed that the prior

standard eradication regimen with combination of doxycycline and trimethoprim-sulfamethoxazole conferred no advantage over use of trimethoprim-sulfamethoxazole alone.[13] Recent MIC studies have shown that prior reports of primary resistance to trimethoprim-sulfamethoxazole being present in over 10% of *B. pseudomallei* isolates from Thailand and other Southeast Asian countries were inaccurate,[186] making trimethoprim-sulfamethoxazole alone now the preferred initial eradication agent of choice for melioidosis (table 2).

In some locations in Thailand amoxicillin-clavulanate has been used for eradication therapy in children and in pregnancy, but in Australia trimethoprim-sulfamethoxazole is the preferred eradication therapy for children and potentially in pregnant women, after the first trimester (risk of neural tube/other congenital defects). Dosing with amoxicillin-clavulanate is problematic and acquired resistance is well documented when amoxicillin-clavulanate or doxycycline are used.[5]

Empirical experience and therapeutic modelling have resulted in melioidosis dosing recommendations for trimethoprim-sulfamethoxazole[199] and amoxicillin-clavulanate[200] that are higher than the standard doses more commonly used with these antibiotics. Consequently, adverse effects are not uncommon with the prolonged course of trimethoprim-sulfamethoxazole required, being reported in up to 40% of patients.[13] In Thailand the drug is usually avoided in patients known to be glucose-6-phosphate dehydrogenase (G6PD) deficient (risk of haemolytic anaemia), although patients are not routinely screened prior to treatment. Rash, gastrointestinal symptoms, hyperkalemia and rising creatinine may necessitate dose modification or cessation of trimethoprim-sulfamethoxazole, with a switch to the alternatives of doxycycline or amoxicillin-clavulanate. Desensitization should be considered for non-severe skin reactions attributed to trimethoprim-sulfamethoxazole.

[H2] Duration of intensive and eradication therapy

Studies from Thailand showed failure of eradication therapy to be associated with poor adherence to therapy, more severe infections (multifocal disease and bacteremia) and duration of eradication therapy of < 8 weeks.[133,201,202] This underpins the recommendations for an eradication phase of 3-6 months (table 2). In recent years lengthening of the duration of intravenous therapy for patients with more severe melioidosis has paralleled the decrease in mortality in those regions where hospital and patient resources make extended use of ceftazidime or meropenem a realistic option. Such prolonged intravenous therapy is supported by a retrospective analysis of the Royal Darwin Hospital melioidosis treatment guidelines, which define the minimum recommended duration of intravenous therapy based on the clinical presentation.[203] The median duration of intravenous therapy for patients in that analysis was around 4 weeks and only 5 (1.2%) patients relapsed. The low current relapse rates in Darwin occur despite patients commonly ceasing the eradication therapy early, frequently missing doses or not taking any of their oral eradication therapy after discharge, with the good outcomes attributed to prolongation of intravenous therapy.[203] Studies are now required to assess whether future guidelines can include options based solely on intravenous therapy and without the need for a prolonged eradication phase.

There is support from case series for selected patients with localized cutaneous disease to be treated with oral trimethoprim-sulfamethoxazole for 3 months without preceding intravenous therapy.[38,204,205] Such a regimen must be restricted to patients who are systemically well, have no signs of sepsis or organ dysfunction, have no underlying risk factors, and have no dissemination of the infection to other sites including regional lymph nodes, all of which should be ruled out by a full melioidosis workup.

[H2] Surgical aspects of therapy

Surgical drainage is usually required for single, large abscesses such as seen in the liver and muscles, but drainage is not necessary or possible for multiple small abscesses in the spleen, liver and kidney. Other internal collections rarely need to be drained as they frequently resolve with medical therapy. Prostatic abscesses usually do require drainage.[206] Septic arthritis usually requires operative drainage and washout and may require repeated procedures. Osteomyelitis can be very extensive when diagnosis and appropriate antibiotic therapy are delayed and such cases usually need aggressive and often repeated surgical debridement of necrotic bone.[207] However, early long bone osteomyelitis without abscess formation and vertebral osteomyelitis without epidural abscess may not require debridement. Mycotic aneurysms require urgent surgery, often with insertion of prosthetic vascular grafts. Lifelong suppressive therapy with trimethoprim-sulfamethoxazole may be indicated for those who have received prosthetic grafts for mycotic aneurysms.

[H2] Critical care therapy

State-of-the-art intensive care management has a major impact in decreasing mortality in patients with melioidosis sepsis and septic shock.[208,209] For these patients, it is advised to follow the Surviving Sepsis Campaign guidelines for the management of sepsis and septic shock.[210]

Granulocyte colony-stimulating factor (G-CSF) has been used empirically in patients with melioidosis septic shock. The rationale is to counteract the functional neutrophil defects thought to be critical in the pathogenesis of severe melioidosis. Early observational data showed a significant improvement in survival with G-CSF, but this was confounded by

concomitant improvements in other aspects of patient management.[211] A subsequent randomized controlled trial in Thailand showed no overall survival benefit with addition of G-CSF;[212] nevertheless, survival was significantly longer in the G-CSF group where there were limited intensive care resources, and adjuvant G-CSF is still used for melioidosis septic shock in some hospitals with state-of-the-art intensive care facilities. Preclinical models have shown that administration of clinically available IL-1 blocking agents can protect mice against *B. pseudomallei* infection.[111,213] This is in line with reports that diabetic patients with melioidosis taking glyburide (glibenclamide), have a lower mortality and attenuated inflammatory responses compared with patients not taking glyburide (box 1).[214] Given the critical role of immune function in melioidosis pathogenesis, patients with melioidosis septic shock are an important cohort for study of newly available immune-modulating therapies for use in sepsis.

[H1] Quality of Life

[H2] Recrudescence and recurrence of melioidosis

Recrudescent melioidosis with return of clinical illness and culture positivity is seen during the treatment period if intravenous therapy is ceased too early, internal collections have not been diagnosed or adequately drained or if oral eradication therapy is not adhered to or is ceased too early. Patients with recrudescence will need hospital admission, intravenous parenteral antimicrobials, and re-investigation for sites of infection.

Subsequent to completion of therapy, recurrent melioidosis may occur due to either relapse of the original infection, as confirmed by bacterial isolate genotyping, or new infection with a different strain.[133,215] With improvements in therapy, relapse is now uncommon, having

decreased from around 10% of cases to well under 5%; new infections in melioidosis survivors are now more common than relapses.[203 13,216,217]

[H2] Underlying diseases and residual deficits

For survivors of melioidosis the main determinant of future health is their underlying risk factors that predisposed to their initial infection. Many of the patients in the Darwin Prospective Melioidosis Study have subsequently died or had substantial and ongoing disability as a consequence of their diabetes, chronic renal disease or malignancy. The notable disability that is melioidosis-specific is residual neurological deficit subsequent to melioidosis encephalomyelitis. While rare, this is particularly problematic when seen in children, with examples ranging from severe residual quadriparesis or severe flaccid paraparesis to persisting isolated foot drop.[38] Limited range of motion, sinus tract formation and joint deformities are also not uncommon in patients with bone and joint infections.[207,218]

[H1] Outlook

[H2] Melioidosis as a neglected disease

Neglected tropical diseases (NTDs) are understudied diseases that which remain endemic in many developing countries around the world.[219] Melioidosis is not included in most lists of neglected tropical diseases, even though it has high mortality rates and is potentially preventable and treatable.[4] No fact sheet about melioidosis is available on the WHO website.[219] Efforts by the international research community are needed to raise awareness of melioidosis within the WHO, regional and local health agencies, as in the general public in endemic areas.

In the past decade, efforts by the international community to prioritize NTDs have resulted in revised public health policies as well as financial support to combat these diseases.[220] In 2015, the International Melioidosis Society (IMS) was formed by melioidosis researchers to raise awareness and knowledge of the disease amongst all stakeholders, and in 2016 a Research Collaboration Network (RCN) was formed to bring the disease to the attention of public health officials and policy makers in melioidosis-endemic countries. An interactive map and disease information are available on the IMS/RCN website (www.melioidosis.info).

[H2] Detection and management of melioidosis in developing countries

Diagnosis of melioidosis relies on the availability and utilization of clinical microbiology laboratories, and awareness of melioidosis among local clinicians and laboratory technicians. The disease is most common amongst the rural poor, who often have limited access to even simple diagnostics. Clinical microbiology laboratories may be underused in many developing countries and not necessarily because of costs.[221] Non-culture-based methods to diagnose bacterial infectious disease are increasingly being encouraged,[222] but they have not been extensively evaluated for melioidosis. Therefore, priority should be given to the establishment of basic clinical microbiology laboratories in endemic areas if melioidosis case ascertainment is to be improved. In addition, education about melioidosis disease and prevention should be provided to both clinicians and laboratory technicians in all tropical low and middle-income countries (LMICs).

To reduce mortality caused by acute melioidosis, availability and affordability of ceftazidime or carbapenems needs to increase. In Thailand, an upper-middle income country, a 14-day course of ceftazidime or carbapenem costs about $60 or $1,080, respectively, and is covered by Thailand's Universal Healthcare Coverage Scheme.[223] Nonetheless, in other LMICs, drugs

could be more expensive, have limited availability or excluded from the country's universal health coverage, and patients frequently cannot afford to pay for the drugs themselves.[224] Studies on how best to allocate and utilize resources for melioidosis in LMICs are urgently needed.

[H2] The One Health Initiative

Since both humans and animals may contract melioidosis, it is essential to implement an approach that can promote effectiveness in addressing the underlying social, human health, zoonotic, and environmental challenges to prevent morbidity and mortality from melioidosis. [225] The "One Health" initiative is a strategy involving interdisciplinary collaborations of health professionals at the local, national, and global level in all aspects of health care for humans, animals and the environment.[226] During the Eighth World Melioidosis Congress in the Philippines in 2016, it was highlighted that melioidosis is a "sapronosis", a disease of animals and humans caused by an environmental organism; however, disease in animals and geographical distribution of distinct human clinical manifestations are not well understood.[225] Thus, adopting the One Health model will promote cooperation and strategic planning between physicians, ecologists and environmental scientists, and veterinarians with the aim of improving health for both humans and animals. Further, interdisciplinary efforts will help address the sapronotic spread of the disease, which will provide valuable information to establish effective melioidosis interventions.

To implement One Health approaches, the melioidosis community needs to be sensitive to resource-poor settings and should leverage partners and broader global public health networks. If possible, the integration of potential technology-based solutions, such as wireless and mobile technologies for health intervention/education delivery as well as the expansion

of the interactive tools currently displayed in the melioidosis website should be utilised. One Health melioidosis initiatives can also be conducive to broader engagement with organizations and individuals with knowledge, ability, and experience in NTDs-like programmatic areas of prevention, surveillance, clinical case management, as well as those with training in economic development, genomics, veterinary sciences, wildlife management, agriculture, molecular biology and bacteriology, ecology, policy, and law.[226] The implementation of this interdisciplinary initiative provides an important opportunity to combine field efforts addressing both endemic and emerging melioidosis but requires effective global health governance to make it operational.[225]

[H2] Advances in the development of a vaccine

In 2015, a Steering Group on Melioidosis Vaccine Development (SGMVD) was formed with the mission of advising research groups and funding agencies about the priority areas to advance different melioidosis vaccine candidates to pre-clinical studies and eventually, human trials.[227] This group has made several recommendations, including the need for standardized animal models and challenge bacterial strains as well as routes of immunization and challenge. Further, this group highlighted the need for distinguishing the requirements of a vaccine targeting the military or other biodefense-related populations from those designed to prevent naturally acquired melioidosis in endemic areas.[227]

Various vaccine formulations have been developed and tested in animal models of melioidosis, resulting in the consensus that live attenuated vaccines induced a more comprehensive immune response and are currently considered the best approach for generating protection against *B. pseudomallei*.[19,228] However, subunit-based vaccination has been shown to provide another feasible alternative because of its increased safety and

potential for large-scale vaccine production. Further, recent experimental evidence indicates that the combination of bacterial polysaccharides (lipopolysaccharide or capsule) with well-defined protein antigens (glycoconjugates), can generate significant protection against *Burkholderia* infections.[228] This rapid advancement in vaccine design and optimization is very promising and several platforms are currently tested simultaneously. However, it is very likely that a multivalent vaccine containing numerous immunogenic bacterial components will be necessary to achieve complete protection, because in addition to a strong antibody response which is necessary for vaccine-induced protection, it is now accepted that both $CD4^+$ and $CD8^+$ T cells are also important in protection against human melioidosis.

A vaccine could be a cost-effective intervention in tropical developing countries, particularly if used in high-risk populations such as diabetics, even if it produced only partial immunity.[229] No melioidosis vaccine is currently available for human use; however,[227,228] a number of vaccine candidates have been shown to provide partial protection against melioidosis or glanders in murine models of infection,[229-231] but few have been tested to date in non-human primates (NHP) or humans.[232]

[H2] Final thoughts

Future questions include to what degree new global environmental sampling studies and improvements in diagnostic microbiology will enhance the understanding of geographical distribution and burden of *B. pseudomallei*, whether whole genome sequencing on a wider scale together with clinical details can provide novel insights into phylogeny and virulence and whether a representative disability-adjusted life year (DALY) metric can be calculated to provide better comparative burden information and pressure on regulating bodies to recognise melioidosis as a neglected tropical disease. More than 80% of diabetic patients live

in low and middle-income countries and the numbers are projected to increase by 55% globally in the next 20 years, with tropical countries facing the brunt of this epidemic.[233] Case clusters of melioidosis have also been associated with severe weather events,[234,235] which, with current estimates of global climate change may become increasingly common in future. This combination could cause a hidden tide of melioidosis, particularly in countries where it has previously been underreported such as India. Further research is required to understand the ecology and pathophysiology better, whilst developing preventative measures. The application of novel techniques to isolates with well characterised clinical and epidemiological metadata may yet provide further insights in melioidosis. A better understanding of the innate and adaptive immune system with new 'omics' based technologies will allow us to further appreciate how *B. pseudomallei* evades immune surveillance and can remain latent for many years. The role of the microbiome is only just beginning to emerge[236] and may offer creative insights in how to tackle the infection *in vivo* and reduce exposure *in terra*. Finally, the current limitations in diagnosis and therapy highlight the need for cheaper effective alternatives and novel drug targets to reduce mortality, relapse and course lengths.

Boxes, Figures and Tables

BOX 1 Diabetes and melioidosis

Recurrent observations over the years have identified a strong correlation between diabetes and *B. pseudomallei* infection, with 23-60% of melioidosis patients being diabetic.[15] Diabetes results in blunted *B. pseudomallei* specific cellular responses during acute infection,[136] resulting in decreased capacity for macrophages to phagocytose and kill, reduced LPS induced generation of CD4 T_{reg} cells and impairment of TLR-mediated MyD88 inflammatory signaling. Dysregulated phosphorylation of NFκB with subsequent non-inactivation of glycogen synthase kinase 3b results in excessive TNF-α and IL-12 production by mononuclear cells, resulting in greater risk of septic shock.[237,238] Furthermore, disease progression and severity in diabetes is exacerbated by loss of effective CD4+ T-cell proliferation which express higher levels of CD152 and function by increased expression of PDL-1 on neutrophils, which also inhibit IFN-γ production.[140] Similar defects have been observed in association with chronic renal disease, excess alcohol consumption and thalassemia.[8,140]

Several studies have demonstrated defects in neutrophil adhesion, chemotaxis and intracellular killing, but not attenuated neutrophil phagocytosis as previously thought. This is explained by decreased opsonisation of bacteria (a pre-requisite for neutrophil uptake) possibly due to glucose affecting the thioester bond of complement C3 and so preventing binding to the bacterial surface.[239] Studies *in vitro* of diabetic leucocytes displayed a reduced rate of glycolysis, which shows reversibility with insulin supplementation. Humoral responses are also poorer and may have an impact on vaccination.[239]

Perhaps paradoxically, however, diabetes was associated with a lower overall mortality in melioidosis patients in NE Thailand, although this was confined to patients who were being treated with glibenclamide[214], which acts as an anti-inflammatory agent by reducing IL-1β secretion accompanied by diminished cellular influx and reduced bacterial dissemination to distant organs[240] in experimental melioidosis. Patients taking glibenclamide prior to admission also have attenuated inflammatory responses.[240] Glibenclamide is an IL-1 monocyte inhibitor via the NALP3 inflammasome complex and was recently shown to reduce neutrophil pro-inflammatory cytokine production by lowering free glutathione and enhancing IRAK-M pathways. This results in reduced IL-1B secretion in a dose dependent fashion.[241,242] In murine models, it did not affect glucose levels or *B. pseudomallei* growth but was associated with reduced pulmonary cellular influx and bacterial dissemination.[240]

Box 2: Genome and virulence factors of *B. pseudomallei*

The complete genome of *B. pseudomallei* is large, consisting of two circular replicons, chromosome 1 (4.07 Mb) and chromosome 2 (3.17 Mb). Chromosome 1 largely encodes core housekeeping functions such as cell wall synthesis, metabolism and motility, while chromosome 2 is enriched for accessory functions involved in condition-dependent adaptation.[243] Within this bipartite structure, horizontal gene transfer provides genetic plasticity, represented by the large metabolic repertoire and intrinsic redundancy of virulence factors such as type three secretion systems (T3SS).[244] The pan-genome shows significant genetic heterogeneity between strains, largely influenced by horizontal gene transfer, recombination and mutations.[245,246]

Protean disease manifestations, chronic disease and recrudescence in patients with melioidosis may be influenced by a highly plastic and consequent variable genome across species.[104,244] Studies also demonstrate that bacterial genetic mutations occur during the infection time course. For example, mutations in variable-number tandem repeats (VNTRs) can occur over short periods of time based on isolates collected two weeks apart from a single patient with acute melioidosis.[244,245] Geographical segregation may also contribute towards clinical manifestations, with region-specific genetic loci reflecting variability in survival and virulence.[247] Mechanisms restricting inter-clade gene flow have been proposed since specific patterns of recombination and accessory gene exchange are rarely seen.[248] Whole-genome tiling array expression data demonstrated that non-coding RNA may play an important role in virulence and host-pathogen interactions.[6]

Phylogenetic analysis demonstrates greater genetic diversity and a clear distinction between isolates from Australia and Asia, supporting the hypothesis that Australia was an early reservoir for the current global *B. pseudomallei* population.[247] Within the endemic zone of Southeast Asia, the Mekong sub-region has emerged as a hotspot for *B. pseudomallei* evolution.[247] Furthermore, an African origin of American isolates is suggested by close ancestry originating between 17-19th century as seen by a monophyly of isolates from Africa and central and South America.[247]

BOX 3 Antimicrobial resistance

B. pseudomallei genome contains several genes encoding Ambler class A, B and D ß-lactamases. The most important of these is located on chromosome 2 (*penA*) and is expressed as a membrane bound lipoprotein secreted by the twin-arginine transport (TAT) system, hydrolyzing most ß-lactams.[8,249]

Acquired resistance during melioidosis treatment is rare. Studies of acquired ß-lactamase resistance (including carbapenems) whilst on therapy identified three distinct phenotypic changes, mainly resulting from *penA* gene mutations: de-repression of chromosomal enzyme, insensitivity to ß-lactamase inhibitors and specific ceftazidime resistance.[8,250] A study focusing on ceftazidime failures in Thai isolates were found to have large segments of chromosome 2 deleted. This common 71 Kb deleted segment contained 3 genes encoding putative penicillin-binding proteins (PBPs), known targets of ß-lactam antibiotics .[251]

B. pseudomallei encodes at least 10 resistance nodulation division (RND) efflux pump systems, spanning both chromosomes, that confer at least partial resistance to six antibiotic classes, including aminoglycosides, fluoroquinolones and tetracyclines.[249] Mutations targeting dihydrofolate reductase (FolA) confer resistance to trimethoprim.[252] *B. pseudomallei* growing as a biofilm on silastic discs revealed cells were viable after 24 h of antibiotic exposure, with up to 200 times the MIC of planktonic bacteria.[8] Inhibition of efflux pumps may lower resistance to ceftazidime and doxycycline in these biofilms.[253]

B. pseudomallei LPS structure (Ara4N moiety) also plays an intrinsic role in resistance to cationic peptides, such as polymyxin B. Additionally, Omp38, an outer membrane porin, is thought to contribute towards ceftazidime and carbapenem resistance.[249] NDM-1 (a lipoprotein carbapenemase) is expressed on the outer membrane of Gram negative bacilli and can be shed in outer membrane vesicles (OMVs), thus constituting a novel resistance dissemination mechanism conferring phenotypic resistance to beneficiary bacteria.[254] *B. pseudomallei* PenA may have a similar purpose.[254] Worryingly, latest reports from isogenic *B. pseudomallei* strains from patients on meropenem have shown MIC creep towards decreased susceptibility.[255]

BOX 4 Coagulation

New insights have enhanced our knowledge of the role of coagulation, fibrinolysis and interplay with inflammation in the pathogenesis of melioidosis (recently reviewed by Kager et al.).[256] Coagulation, as well as the fibrinolytic system, becomes activated while anticoagulant pathways are downregulated.[257,258] Levels of protein C, protein S and anti-thrombin are all decreased.[257,259] High TATc/PAPc ratios indicate a predominance of pro-coagulant mechanisms in melioidosis and elevated levels of soluble endothelial protein C receptor (EPCR) on hospital admission are associated with increased mortality. Furthermore, plasminogen activator inhibitor type 1 (PAI-1) deficient mice (which have decreased fibrin deposition) show heightened susceptibility to *B. pseudomallei*.[260] Activated protein C (APC) and the protein C system appear to play a bidirectional role, with a minimal amount of APC required to support an appropriate antibacterial host response.[261] Interestingly, the cytoprotective effects of APC are responsible for the protective phenotype. The α2-Antiplasmin (A2AP), a major inhibitor of fibrinolysis, serves as a protective mediator during experimental melioidosis by limiting bacterial growth, inflammation, tissue injury, and coagulation.[262] Urokinase-type plasminogen activator receptor (uPAR) which is also known to play a key role in fibrinolysis, has a significant role in defence against melioidosis by facilitating the migration of neutrophils to site of infection and subsequently enabling the phagocytosis of *B. pseudomallei* further underlying the bidirectional role between coagulation and inflammation in melioidosis.[256]

FIGURE 1: Milestones in the history of melioidosis.

1911 – Melioidosis first recognized by British doctor Alfred Whitmore and his assistant C.S. Krishnaswami in Burma

1921 - Name melioidosis coined by Stanton and Fletcher

1927 – First case (human) reported in South Asia (Sri Lanka)

1932 – 83 cases reported in South and Southeast Asia with 98% mortality (81/83)

1936 – First case (pig) reported in Africa (Madagascar)

1937 – Soil and water identified as habitat of *B. pseudomallei*

1947 – First case (human) reported in Central America (probably Panama)

1949 – First case (sheep) reported in Australia

1967-1973 – Among American soldiers in Vietnam, term 'Vietnamese time-bomb' coined for the reactivation of latent infection, inhalation suspected as a route of infection in about 50 helicopter crewmen, and a total of 343 melioidosis cases were reported

1982 – First evidence of *B. pseudomallei* (in soil) in South America (Brazil)

1989 – Ceftazidime reported to halve mortality of melioidosis (from 74% to 37%)

1992 – Transfer of species *to Burkholderia pseudomallei* *

2002 – *B. pseudomallei* classified as 'Category B' Critical biological agent for public health preparedness

- Live attenuated vaccine (2D2) developed in murine models

2003 – MLST scheme for *B. pseudomallei* developed

2004 – First whole genome sequence of *B. pseudomallei*

2012 – *B. pseudomallei* classified as Tier 1 select agent by US CDC

2014 – MERTH study supports TMP-SMX alone as oral eradicative treatment

2016 – Modelling study predicts 89,000 people dying of melioidosis yearly

2016 – Whole genome sequencing links *B. pseudomallei* from air samples to epidemiologically matched human case

* Over the past century, the organism has been renamed many times; names have included *Bacterium* (or *Bacillus*) *whitmori, Malleomyces pseudomallei, Loefflerella pseudomallei, Pfeiferella pseudomallei, Pseudomonas pseudomallei,* and *Burkholderia pseudomallei*.

FIGURE 2. *B.pseudomallei* and infection resulting from common routes of entry

FIGURE 3 Estimated mortality and reported cases of melioidosis.[263]

Only Australia, Brunei Darussalam and Singapore have national surveillance data for melioidosis that are comparable to the estimates. The model predicted that there could be about 420 melioidosis cases in Lao PDR per year,[263] and recent evidence shows that there were more than 100 culture-confirmed melioidosis cases each year at Mahosot Hospital alone in Laos between 2010 to 2015.[264] This supports the estimates. However, the model predicted that there could be about 20,038 melioidosis cases in Indonesia per year, while there have been only 22 reported cases in the country.[263] A large difference between predicted cases and observed cases is also observed in China, India, Bangladesh, Nigeria and Brazil.[263] This could be due to limitations of the model, underuse of clinical microbiology laboratories,[221] lack of awareness of melioidosis and poor disease reporting systems.

FIGURE 4 Schematic model of host-pathogen interactions and pathophysiology of melioidosis

B. pseudomallei has the ability to invade, survive and replicate in many cell types with upregulation of putative virulence factors at various stages to overcome host defense mechanisms. AHL quorum sensing machinery is used to co-ordinate attack against the host environment and biofilm formation. T3SS encodes protein effectors necessary for invasion and phagosome escape, with entry aided by flagella, LPS, pilA and BoaA/B. Passive uptake by phagocytic cells also occurs. Upon cell entry *B. pseudomallei* quickly escapes phagosomes by lysing the membrane and making use of T3SS, T6SS and T2SS. Specifically, TssM inhibits iNOS and IFN-β production by a deubiquitinating pathway. Metabolic flexibility (resisting oxidative stress), resistance to antimicrobial resistant peptides and ecotin production allow the bacterium to survive within an acidic endocytic environment. BopA and BipD further blocks sequestration in phagosomes and prevents LAP associated autophagy once free in the cytosol. Once free in the cytoplasm, *B. pseudomallei* replicates freely and can also release BLF-1, irreversibly inhibiting RNA-helicase activity. Within the cytoplasm *B. pseudomallei* induce formation of actin based membrane protrusions and can mobilise by continuous nucleation of host cell actin at polar ends (regulated by BimA), facilitating spread to neighbouring cells. This results in cell fusion and MNGC formation. T6SS and Hcp1 are essential to this process. TLRs located on cell surfaces work together in recognizing PAMPS (such as flagellin and T3SS proteins BopE) and mediating NFκB induced activation of immune response, releasing pro-inflammatory cytokines IL-1β and IL-18. MyD88 serves as a central TLR adaptor molecule and IRAK-M is a negative regulator of this TLR cascade. To avoid macrophage killing, *B. pseudomallei* fails to activate genes downstream of the TRIF-dependent TLR pathway, such as IFN-γ and iNOS, which is linked to paucity of activation of IFN-γ regulatory factor 1 (IRF-1)

and STAT-1 transcription factors, necessary for increased iNOS expression. Intracellular inflammasome receptors such as NOD like receptors NLRC4 and NLRP3 recognise bacterial virulence factors and DAMPS, triggering caspase-1 mediated pyroptosis (cell death limiting bacterial growth) and further release of IL-1β and IL-18. IL-18 further ensures protective IFN-γ production (mainly from NK cells). Neutrophils, dendritic cells and leukocytes are recruited towards site of infection, with complement and coagulation cascade activations heralding full blown sepsis.

FIGURE 5 Distribution of organ involvement in melioidosis patients

~~**FIGURE 6: Management algorithm for melioidosis**~~

1179 **TABLE 1 Selected Virulence factors of *B. pseudomallei*†**

Gene	Antigen	Function	Ref.
Adherence			
pilA	Type IV pilin subunit protein PilA	Mediates temperature-dependent adherence and formation of microcolonies in some *B. pseudomallei* strains, intracellular motility	[48,55]
boaA/B	Adhesins	T5SS autotransporters which also play a role in cell attachment and possibly intracellular replication	[59,265,266]
bpaC	Adhesin	T5SS trimeric autotransporter adhesin. Protects from complement killing and involved in cell attachment, which may be cell specific (e.g. ciliated mucosal epithelial cells)	[49,266]
fliC	Flagellin structural component	Required for flagella assembly (polar tuft of 2-4 flagella allows temperature independent motility) and involved in cell adherence	[267,268]
Invasion			
bopE	T3SS effector (guanine nucleotide exchange factor)	Targets Cdc42 and Rac1, inducing actin rearrangements hence aiding cell invasion	[60]
bipB/C/D	T3SS translocator	Involved in phagolysosome survival, escape and cell invasion	[52,67]
irlR	Two-component response regulator	Mutants displayed reduced invasion	[63]
Endocytic escape			
bopA	T3SS effector	Involved in phagosome membrane disruption and avoidance of autophagy	[83,84]
bsaQ	T3SS structural component	Involved in phagosomal escape, cell invasion and plaque formation	[51,269]
bsaZ	T3SS structural component	Implicated in vacuolar escape and intracellular replication	[52,270]
bsaU	T3SS structural component	Involved in escape and early onset activation of caspase-1 pathway in macrophages	[122,271]
CHBP (*cif* homolog)	T3SS structural component (ATP/GTP binding protein)	Delays host cell maturation, arresting cycle in G2/M and impeding apoptosis	[16,49,67,272]
Intracellular survival			
purM/N	Phosphoribosyl aminoimidazole formyltransferase/synthetase	Purine biosynthetic pathway. KO models show decreased intracellular replication	[48,271]
sodC	Superoxide dismutase	This and other enzymes (KatG, AhpC and DNA binding protein DpsA) mediate resistance to oxidative stress	[74-77]
rpoE	RpoE	Biofilm formation, heat stress response via RpoH regulated heat-shock proteins, oxidative and osmotic stress. Mutants show reduced intracellular survival in macrophages	[50,273]
virAG	Regulates T6SS transcription	Sensor and histidine kinase. Upregulated at acidic pH, affecting T6SS secretion process.	[16,244]
rpoS	Global regulatory factor	Suppress iNOS by upregulating SOCS3 and CIS cytokines. May play a role in regulating genes involved in macrophage fusion	[72,274]
Actin based motility			
bimA	T5SS autosecreted effector	Escape from phagosome, autotransporter, actin tail formation. Encephalomyelitis strongly correlated with	[86,87,89]

		$BimA_{Bm}$ allele	
MNGC formation			
hcp1	Inner tube T6SS-1	Role in cell fusion, macrophage cytotoxicity. Induces IL-10 and TGF-β	[275-278]
vgrG-5	T6SS tail-spike tip	VgrG5 functions in MNGC formation. Functionally conserved across *Burkholderia* spp.	[279]
Others			
tssM	Deubiquitinase	T2SS (part of Gsp) effector. Targets TRAF3, TRAF6 and IκBα to inhibit type I IFN and NFκB pathways	[53,280]
wcb cluster	CPS	There are four CPS structures, CPSI-IV, *wcb* transcribes CPS-I. Protects from C3b complement and NHS, CPSIII has environmental role. Biofilm production, not essential for survival but contributes towards persistent infection/latency	[281-283]
waaF, lytB and other in the operons	LPS	3 serotypes, smooth type A predominates, confers resistance to NHS and from cationic peptides. Reduced minimal pyrogenic lethal toxicity and macrophage activation. Length, number and position of fatty acyl chains can affect LPS bioactivity and vary between virulent strains	[50,118,119,284]
luxI/R homologs	N-acyl-homoserine lactone (AHL) quorum-sensing (QS)	Mediated by AHLs and a second system using HMAQ. Upregulates transcription of genes simultaneously within a population involved in colonisation, longer survival and higher LD_{50}. LuxI and R have also been linked to regulation of siderophore synthesis	[285,286]
BLF1 (*Burkholderia* lethal factor-1)	Glutamine deamidase	Like *E. coli* cytotoxic necrotising factor. Irreversibly interferes with initiation of translation by inactivating eIF4A and thereby recruitment of 40S ribosomal subunit thus protein synthesis. Cell cytoskeleton alteration and cell death. Concentrations low as $2.5x10^{-7}M$ can cause effect with molecular turnover like that of ricin	[287,288]
Multiple genes upregulated in tandem	Morphotype switching	Seven morphotypes, wrinkled type 1 predominates. Strain differences in colony morphology phenotypically lead to changes in biofilm production, secreted enzymes and motility, hence influencing intracellular survival, lethality and persistence.	[289]

†*This is not a comprehensive list of all B. pseudomallei virulence factors but have been selected as a well-studied representative sample involved throughout the life cycle of B. pseudomallei. A number of other biologically active molecules such as proteases, lipases, haemolysins and siderophores are also secreted via the Gsp.*

Abbreviations: Cdc42, cell division control protein 42 homolog; Rac1, Ras-related C3 botulinum toxin substrate 1; MNGC, multi-nucleated giant cell; iNOS, inducible nitric oxide synthase; SOCS3, suppressor of cytokine signaling 3; CIS, cytokine inducible src homology 2 containing protein; Gsp, general secretory pathway; NHS, normal human serum; CPS, capsular polysaccharide; LPS, lipopolysaccharide; HMAQ, 4-hydroxy-3-methyl-2-alkylquinolone signaling molecules; LD_{50}, lethal dose 50%; eIF4A, eukaryotic initiation factor 4A.

TABLE 2 Antibiotic therapy for treatment of melioidosis‡

Initial intensive therapy:
ceftazidime (wards) 2 g (child: 50 mg/kg up to 2 g) IV, 6-hourly for at least 10-14 days ***OR*** **meropenem** (ICU) 1 g (child: 25 mg/kg up to 1 g) IV, 8-hourly for at least 10-14 days
For neurological melioidosis meropenem is the preferred initial IV therapy and the meropenem dose is doubled to 2 g (child: 50 mg/kg up to 2 g) IV, 8-hourly
For neurological melioidosis, osteomyelitis and septic arthritis, genitourinary infection including prostatic abscesses, and skin and soft tissue infections, consider adding trimethoprim+sulfamethoxazole from commencement of therapy in the eradication doses as below.
Prolonged IV therapy (4 to 8 weeks or longer) is recommended where possible for complicated pneumonia, deep-seated infection including prostatic abscesses, neurological melioidosis, osteomyelitis and septic arthritis.[180,290]
Eradication therapy: *(Required after the initial intensive therapy)*
trimethoprim+sulfamethoxazole child 6+30 mg/kg up to 240+1200 mg; adult 40-60kg, 240+1200 mg; >60kg, 320+1600 mg orally, 12-hourly for at least a further 3 months (6 months for neurological melioidosis and osteomyelitis) ***PLUS*** **folic acid** 5 mg (child: 0.1 mg/kg up to 5 mg) orally, daily for at least a further 3 months

‡The longer IV duration recommendations are derived from Australian studies[15,203] and apply to resource-rich countries. The current reality for many in melioidosis-endemic regions is that such prolonged intravenous therapy is either not available or not affordable. Nevertheless a minimum of 10 days of IV therapy is recommended for all cases of melioidosis except for those with localized skin disease without sepsis (see text).[180,187] This treatment guidance is consistent with the most up-to-date recommendations by International Melioidosis Society (http://www.melioidosis.info).

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