



Published in final edited form as:

*Nat Rev Dis Primers*. ; 4: 17107. doi:10.1038/nrdp.2017.107.

## Melioidosis

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### Abstract

*Burkholderia pseudomallei* is a Gram-negative environmental bacterium and the aetiological agent of melioidosis, a life-threatening infection that is estimated to account for ~89,000 deaths per year worldwide. Diabetes mellitus is a major risk factor for melioidosis, and the global diabetes pandemic could increase the number of fatalities caused by melioidosis. Melioidosis is endemic across tropical areas, especially in southeast Asia and northern Australia. Disease manifestations can range from acute septicaemia to chronic infection, as the facultative intracellular lifestyle and virulence factors of *B. pseudomallei* promote survival and persistence of the pathogen within a broad range of cells, and the bacteria can manipulate the host's immune responses and signalling pathways to escape surveillance. The majority of patients present with sepsis, but specific clinical presentations and their severity vary depending on the route of bacterial entry (skin penetration, inhalation or ingestion), host immune function and bacterial strain and load. Diagnosis is based on clinical and epidemiological features as well as bacterial culture. Treatment requires long-term intravenous and oral antibiotic courses. Delays in treatment due to difficulties in clinical recognition and laboratory diagnosis often lead to poor outcomes and mortality can exceed 40% in some regions. Research into *B. pseudomallei* is increasing, owing to the biothreat potential of this pathogen and increasing awareness of the disease and its burden; however, better diagnostic tests

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Author contributions

Introduction (H.S.V. and W.J.W.); Epidemiology (D.L. and D.A.B.D.); Mechanisms/pathophysiology (H.S.V., W.J.W., A.G.T. and S.J.P.); Diagnosis, screening and prevention (D.A.B.D., D.L. and B.J.C.); Management (B.J.C.); Quality of life (B.J.C.); Outlook (A.G.T., H.S.V., D.A.B.D., D.L. and W.J.W.); Overview of Primer (all authors).

Competing interests statement

D.A.B.D. acted as a consultant to Soligenix, Inc. All other authors declare no competing interests.

are needed to improve early confirmation of diagnosis, which would enable better therapeutic efficacy and survival.

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Melioidosis is an infectious disease caused by the environmental Gram-negative bacterium *Burkholderia pseudomallei*. First recognised in 1911 (REF. 1) (FIG. 1), the organism is commonly found in the rhizosphere (the layer of soil directly influenced by root secretions and soil microorganisms)<sup>2</sup> and surface groundwater of many tropical and subtropical regions<sup>3,4</sup>, and can infect humans and a wide range of animals.

Naturally acquired infections in humans and animals results from exposure through broken skin, inhalation or ingestion of *B. pseudomallei*<sup>5</sup>; certain environmental conditions, such as tropical storms and specific occupations (for example, rice farming), are known to increase the risk of exposure<sup>6</sup>. *B. pseudomallei* infection can be acute, chronic or latent, although infection usually results in subclinical disease as the majority of immunocompetent individuals can clear the infection. Only those individuals with *B. pseudomallei* infection who develop clinical symptoms (either acute or chronic) are considered to have melioidosis.

Most cases of melioidosis (85%) result in acute infections from recent bacterial exposure<sup>7</sup>. The majority of patients with acute melioidosis present with sepsis (a life-threatening, dysregulated, systemic inflammatory and immune response that can cause organ dysfunction) with or without pneumonia, or localized abscesses, regardless of the route of infection. However, the presence of nonspecific signs and symptoms can often hinder the diagnosis and management of melioidosis, which has been nicknamed ‘the great mimicker’ (REF. 8). Chronic melioidosis is defined as a symptomatic infection that lasts >2 months, and it occurred in 11% of individuals infected with *B. pseudomallei* in a 20-year prospective Australian study<sup>7</sup>. The host’s immune response to acute infection is both humoral (involving cytokine release, especially interferon- $\gamma$  (IFN $\gamma$ )) and cell-mediated, and can completely eradicate or control the infection in most immunocompetent individuals. An unknown percentage of people exposed to *B. pseudomallei* can develop a latent infection (that is, the infection is asymptomatic and the pathogen is not cleared); activation from latency has been estimated to account for <5% of all melioidosis cases<sup>7</sup>, but may result in infection becoming apparent many years after exposure.

The case fatality rate of melioidosis is 10–50%<sup>6</sup>. Of the individuals who survive acute melioidosis, 5–28% experience recurrent infection, which could be due to recrudescence (that is, recurrence) of the original strain, which was not completely cleared and persisted in a dormant state, or reinfection with a different strain following re-exposure<sup>6,9–11</sup>. Approximately 80% of patients have known risk factors, mainly diabetes mellitus<sup>12</sup> (BOX 1). The host-pathogen interplay is complicated by the tropism of the pathogen for a wide variety of cells and its ability to subvert and avoid the host innate immune response<sup>13</sup>.

*Burkholderia mallei* is a host-adapted (mainly causing infections in animals) species that originally derived from *B. pseudomallei* following substantial genome reduction (also known as genome degradation). *B. mallei* is extremely infectious, mainly to solipedes (mammals that have a single hoof on each foot; for example, horses) but can occasionally infect humans. *B. mallei* is the aetiological agent of glanders, a disease with similar

manifestations to melioidosis. The US Centers for Disease Control and Prevention (CDC) have classified *B. pseudomallei* and *B. mallei* (which was used as a biological weapon in World War I)<sup>6</sup> as tier 1 select agents because of their biothreat potential (tier 1 select agents present “the greatest risk of deliberate misuse with the most significant potential for mass casualties or devastating effect to the economy, critical infrastructure; or public confidence”)<sup>14</sup>. No vaccine for either is currently available<sup>15,16</sup>, which further exacerbates concerns of a possible emerging public health threat.

This Primer summarizes the state of the field in melioidosis research, focusing on epidemiology, pathophysiology (including host-pathogen interactions), diagnostics, screening, prevention and clinical management. In the Outlook, we explore future directions of research in the omics and cutting-edge immunology era, argue whether melioidosis should be recognized as a neglected tropical disease and discuss whether a viable vaccine is on the horizon.

## Epidemiology

### ***B. pseudomallei* in the environment**

*B. pseudomallei* is well-known to be present in soil and surface water in southeast Asia and northern Australia; however, case reports of melioidosis and predictive modelling studies suggest that it is probably widely present in many countries across the tropics (BOX 2).

A consensus guideline for soil sampling for *B. pseudomallei* was proposed in 2013 with the goal of elucidating the global distribution of the bacterium<sup>17</sup>. *B. pseudomallei* is most abundant in soil at depths of 10 cm from the surface<sup>17</sup>; however, during the rainy season it can move from deeper soil layers to the surface, where it can then multiply<sup>17</sup>.

*B. pseudomallei* can survive in extreme conditions, such as in distilled (without nutrients) water (for 16 years)<sup>18</sup>, nutrient-depleted soil<sup>19</sup> or desert environments<sup>20</sup>. Outbreaks of melioidosis from contaminated, unchlorinated water supplies have been reported in the Northern Territory, Australia<sup>21</sup>, and have been associated with chlorination failure (that is, insufficient addition of chlorine to the water) in Western Australia<sup>22</sup>. *B. pseudomallei* is also commonly found in unchlorinated water supplies and drinking water in rural areas in Thailand<sup>23</sup>. Nosocomial (originating in a hospital) infections have been attributed to *B. pseudomallei*-contaminated wound irrigation fluid, antiseptics and hand wash detergent<sup>24,25</sup>.

*B. pseudomallei* has rarely been detected in air. Aerosolized bacteria were first isolated in 1989 (REF. 26), and in 2015, *B. pseudomallei* DNA was detected in filtered air using quantitative PCR<sup>27</sup>. Whole-genome sequencing linked the bacteria found in an air isolate to the clinical isolate from a patient with mediastinal melioidosis (that is, with infection of the midline anatomical structures or connective tissue of the chest)<sup>28</sup>. Penetration through the skin, ingestion and inhalation are all important routes of infection with environmental *B. pseudomallei*<sup>5</sup>. Reported neonatal cases were probably caused by mother-to-child transmission (vertical transmission or breastfeeding)<sup>29</sup>, healthcare-associated infection<sup>29</sup> or community-acquired infection<sup>29</sup>. Melioidosis is not contagious and human-to-human transmission has rarely been reported<sup>6</sup>.

## Global burden of melioidosis

A 2016 modelling study estimated that there are ~165,000 cases of melioidosis in humans per year worldwide, of which 89,000 (54%) are estimated to be fatal<sup>3</sup> (FIG. 2). This study highlights that underdiagnosis and under-reporting of melioidosis are a major issue, especially on the Indian subcontinent, where 44% of cases were predicted to occur (predicted incidence for India, Indonesia and Bangladesh are ~52,500, ~20,000 and ~16,900 cases per year, respectively). However, only ~1,300 cases were reported per year worldwide since 2010, which is <1% of the estimated annual incidence<sup>3</sup>. Melioidosis is prevalent in the Northern Territory, Australia, and northeast Thailand, where the annual incidence is up to 50 cases per 100,000 individuals<sup>4,7</sup>, and the emergence of melioidosis in areas where it was previously absent, for example, in northeastern Brazil, could be explained in part by the increasing recognition of this disease, owing to increased awareness and improved diagnostics<sup>30</sup>. Although reports of *B. pseudomallei* isolation from soil and animals in equatorial Africa are limited, they suggest that melioidosis is widely distributed across this region<sup>31–33</sup>. For example, Nigeria is predicted to have an incidence of ~13,400 cases per year, which is comparable to incidences observed in endemic regions such as India, Indonesia and Bangladesh<sup>3</sup>.

The predicted mortality from melioidosis is comparable to that of measles (95,600 individuals per year) and higher than that for leptospirosis (50,000 individuals per year) and dengue infection (12,500 individuals per year), which are diseases that are considered of high priority by many international health organizations<sup>3</sup>. Melioidosis can affect all age groups. In prospective studies in Australia and Thailand, the median age of patients with melioidosis was 50 years, with 5–10% of patients of <15 years of age<sup>7,34,35</sup>.

## Risk factors

The most common risk factor predisposing individuals to melioidosis is diabetes mellitus, which is present in >50% of all patients with melioidosis worldwide<sup>35,36</sup> (BOX 1). Individuals with diabetes mellitus have a 12-fold higher risk of melioidosis after adjustment for age, sex and other risk factors<sup>35,36</sup>. Other known risk factors include exposure to soil or water (especially during the rainy season), male sex (probably because of a greater risk of environmental exposure), age of >45 years, excess alcohol consumption and liver disease, chronic lung disease, chronic kidney disease and thalassaemia (which probably causes neutrophil dysfunction due to iron overload)<sup>6,37</sup>. Prolonged steroid use and immunosuppression can also predispose individuals to infection. Nonetheless, >80% of paediatric patients<sup>34,38</sup> and ~20% of adult patients have no recognized risk factors<sup>35,36</sup>. Melioidosis in adults who have no risk factors generally occurs in those who have been exposed to a high bacterial load, for example, by aspiration of surface water<sup>39</sup>. Zoonotic transmission to humans resulting from contact with livestock is extremely rare; only three possible cases have been reported in Australia<sup>6</sup>.

## Mechanisms/pathophysiology

*B. pseudomallei* is an opportunistic, facultative intracellular, motile saprophyte (an organism that obtains its energy from decaying organic matter) that possesses a remarkable intrinsic

array of virulence factors (TABLE 1) and broad antimicrobial drug resistance (BOX 3). *B. pseudomallei* is highly adaptable, a property that enables it to generate a variety of clinical manifestations, depending on the infected tissue, and to maintain a survival advantage in infected hosts and the environment<sup>40</sup>. Numerous studies have increased our insights into the pathogenesis of *B. pseudomallei* infection<sup>13,41–44</sup> (FIG. 3).

## B. *pseudomallei* infection

*B. pseudomallei* first enters and replicates in epithelial cells of the mucosal surface or broken skin, depending on the route of entry, and then spreads to various cell types. However, the mechanisms of cell invasion and replication are largely similar and are, therefore, discussed collectively (unless otherwise specified).

### Epithelial attachment and cell invasion.

*B. pseudomallei* possesses multiple secretion systems, which are evolutionary apparatuses that enable the transport of proteins across cellular membranes in response to the environment and, therefore, host cell invasion. The secretion systems are classified depending on their structure, function and specificity. The type III secretion system (T3SS) comprises a molecular syringe (a structure made of a filamentous needle to translocate effector proteins into the surrounding milieu or cells) that is deployed on close contact with host cells<sup>45,46</sup>, T2SS is widely distributed in Gram-negative bacteria<sup>47</sup> and T5SS secretes autotransporter proteins, which are usually bound to the outer membrane through some adhesin-like proteins<sup>48</sup>.

Attachment to human pharyngeal epithelial cells was initially thought to be mediated by capsular polysaccharides<sup>49</sup> and type IV pili (hair-like structures on the bacterial surface)<sup>50</sup>. However, internalization into a cell line of human alveolar basal epithelial cells was increased in acapsular mutants compared with wild-type *B. pseudomallei*<sup>51</sup>. Furthermore, the type IV pilin protein PilA (encoded by *pilA*), a subunit type IV pili needed for adhesion to epithelial cells, could also have a role<sup>52</sup>.

Flagellar motility favours close contact with protective mucosal linings, but flagella are probably not a major adhesin for mammalian cells<sup>42,53</sup>. Two T5SS adhesin proteins, BoaA and BoaB (TABLE 1), can enhance adherence. However, double *boaA* and *boaB* knockouts show residual binding, indicating that multiple adhesins are required for cell adhesion<sup>54</sup>. Guanine nucleotide exchange factor BopE, a T3SS effector, causes rearrangement of the host actin cytoskeleton (membrane ruffling) and facilitates ingress<sup>55</sup>, and BsaQ (a conserved inner membrane T3SS protein) mutants displayed a 30% reduction in invasion<sup>42</sup>, suggesting that multiple T3SS effectors mediate cell invasion.

Host factors also play a part in epithelial attachment. Protease-activated receptor 1 (PAR1, which belongs to the subfamily of G protein-coupled receptors) is expressed on several cell types (for example, endothelial cells, platelets and monocytes) and promotes *B. pseudomallei* cell invasion, growth and dissemination<sup>56</sup>. However, interestingly, PAR1 inactivation had no effect on *B. pseudomallei*-associated mortality in mouse models, whereas it delayed time to death when the mice were infected with pneumococci<sup>56</sup>.

### Intracellular survival and replication.

*B. pseudomallei* can invade and propagate in both phagocytic and non-phagocytic cells<sup>57,58</sup>; the bacteria replicate intra-cellularly, cause lysis or spread to and infect adjacent cells. This process causes acute symptoms, which can vary depending on the tissue or organ infected.

Following endocytosis, *B. pseudomallei* can be seen in endocytic vesicles and later within the cytoplasm where it replicates<sup>59,60</sup>. The vesicles then fuse with lysosomes and acidify rapidly<sup>61</sup>. The T3SS is crucial for vesicle escape before the bacteria can be degraded, as multiple strains mutant for T3SS proteins showed downstream effects, including reduced formation of the actin tail (a comet-like filamentous tail made using actin molecules from the host that the bacteria use for intracellular motility), intracellular survival, cytotoxicity and intercellular spread<sup>46,62–64</sup>. Survival within the endocytic vesicle is aided by an ecotin (a periplasmic serine protease inhibitor) homologue, which is involved in resisting degradation by lysosomal enzymes<sup>65</sup>.

*B. pseudomallei* can multiply within phagocytes (including neutrophils, monocytes and macrophages) without activating a bactericidal response<sup>57,58</sup>. Despite detection of lysosome fusion within *B. pseudomallei*-infected macrophages (suggesting that degradation of the pathogens can occur to some extent), proliferation of the surviving bacteria ultimately overwhelms the macrophage<sup>66</sup>. However, macrophages activated by IFN $\gamma$  (which mediates the immune response to intracellular pathogens) display improved killing of *B. pseudomallei*, probably via increased activation of inducible nitric oxide synthase (iNOS)<sup>67</sup>.

In fact, bacterial killing is predominantly mediated by reactive nitrogen intermediates and reactive oxygen species (ROS)<sup>67</sup>. Consequently, an important mechanism of *B. pseudomallei* pathogenesis is to suppress iNOS production by upregulating two negative regulatory cytokines: suppressor of cytokine signalling 3 (SOCS3) and cytokine-inducible SH2-containing protein (CIS)<sup>68,69</sup>. Superoxide (O<sub>2</sub><sup>-</sup>) and H<sub>2</sub>O<sub>2</sub> degrading enzymes have been associated with mediating *B. pseudomallei* resistance to oxidative stress<sup>70–73</sup> (TABLE 1).

### Evasion of autophagy and cell lysis.

*B. pseudomallei* may trigger autophagy by a T3SS-dependent process that involves the activation of nucleotide-binding oligomerization domain-containing protein 2 (NOD2, an intracellular pathogen recognition receptor)<sup>74</sup>, resulting in bacterial killing<sup>75</sup>. However, the exact role of NOD2 might not be clearcut, as another study shows that NOD2 promotes the upregulation of SOCS3 (REF. 76). Hence, the mechanisms by which NOD2 leads to containment of *B. pseudomallei* are probably not mediated by cytokine suppression in murine models. Interestingly, polymorphisms in the *NOD2* region are associated with susceptibility to melioidosis<sup>74</sup>. However, the effectiveness of autophagy evasion is influenced by the expression of the T3SS effector protein Bop A. Loss of Bop A (as suggested by its increased colocalization with microtubule-associated proteins 1A/1B light chain 3B (LC3; also known as MAP1LC3B), an autophagy marker protein) leads to a substantial delay in efficient endosome escape, contributing towards reduced virulence<sup>77</sup>. T3SS probably plays a crucial part in the evasion of autophagy, as bacteria with mutant

BopA are taken up by autophagic vesicles more efficiently and have decreased intracellular survival<sup>78</sup>. However, the complete mechanisms of autophagy escape remain to be defined.

*B. pseudomallei* cytotoxicity for certain cell types is also strain-dependent: some strains cause macrophage apoptosis<sup>60</sup>, some strains cause pyroptosis (an inflammatory form of caspase-1-dependent cell lysis)<sup>63</sup> and others cause neither<sup>57</sup>. Macrophage lysis could represent an escape mechanism for *B. pseudomallei* once a threshold bacterial load has been reached<sup>44</sup>. By contrast, apoptosis and degradation of infected neutrophils by macrophages is delayed in melioidosis, favouring bacterial survival<sup>79</sup>.

### Intercellular and secondary spread.

Intercellular spread of *B. pseudomallei* is facilitated by membranous protrusions (generated by bacteria-induced rearrangements of the cytoskeleton) formed by the host cell that extend into neighbouring cells, through which bacteria travel by actin-mediated motility<sup>60,80</sup>. The autotransporter BimA interacts with monomeric actin at the tail-end of the bacteria, where polymerization occurs<sup>81</sup>. Nerve root translocation and migration along infected neurons until *B. pseudomallei* reaches the central nervous system has been supported by animal studies<sup>82</sup> and linked especially to the minority bacterial genotype carrying *BimA<sub>Bm</sub>* (*B. mallei*-like BimA) that is found mainly in Australia<sup>83</sup>. Such cell-to-cell spread occurring along nerve roots could explain the melioidosis encephalomyelitis syndrome (inflammation of the brain and spinal cord), with brainstem disease following nasal or throat infection, and myelitis (inflammation of the spinal cord) following infection through the skin on the limbs<sup>34</sup>.

Intercellular spread results in cell fusion and the formation of multinuclear giant cells (MNGCs)<sup>84</sup>, a hallmark of melioidosis<sup>44</sup>. Eventual death of MNGCs results in the formation of plaques (one or more MNGCs lyse, leaving a clear zone surrounded by a ring of fused cells) and subsequent damage to host cells, which may serve as a nidus for further *B. pseudomallei* replication or latent or persistent infection<sup>85</sup>.

As well as direct cell-to-cell spread, *B. pseudomallei* can also spread to the bloodstream, causing sepsis, and infect antigen-presenting cells, which then can transport the bacteria to the lymphatic system and contribute to dissemination of infection to secondary sites. However, the exact mechanism of secondary spreading remains elusive. Bacteria also remain viable in dendritic cells, inducing maturation and trafficking to secondary lymphoid organs<sup>86</sup>.

### Latent or persistent infection.

*B. pseudomallei* can remain latent for extended periods before immunosuppression or other host stress responses reactivate bacterial proliferation and melioidosis develops. Reported latency periods have ranged from 19 years to 29 years<sup>87–89</sup>, indicating that *B. pseudomallei* can enter a dormant state and evade immune surveillance<sup>44</sup>. Neither the site (tissue or subcellular level) of latency nor the mechanisms by which *B. pseudomallei* remains undetected are clear<sup>90</sup>. By contrast, high antibody titres detected in patients years after an episode of acute melioidosis suggest continuous exposure or covert sequestration (bacteria hiding in cryptic sites with downregulation of products)<sup>91</sup>. *B. pseudomallei* has been found within the nucleus, which could potentially act as a persistence site for later

recrudescence<sup>92</sup>. Strain variability or small colony variants could also play a part in determining whether latent or persistent infection is established<sup>93</sup>.

Some *B. pseudomallei* persistence (and possibly latency) factors have been characterized, including toxin-antitoxin systems (composed of a toxin (protein) and its cognate antitoxin (a protein or non-coding RNA)), metabolic enzymes and adaptive mutations<sup>94</sup>. By entering a slower growth rate, toxin-antitoxin systems enable bacteria to survive under stressful environments, whereas small colony variants can shift to an acid-tolerant state to survive in abscesses<sup>95,96</sup>. Furthermore, the host's immune response and selection pressure of antibiotics can contribute to selecting resistance patterns that can also facilitate the establishment of persistent infection (BOX 3). Multiple genotypes have been identified within a single infection episode, which at least partly results from genetic adaptation to the human host, including inactivation of virulence and immunogenic factors and deletion of pathways involved in environmental survival<sup>97</sup>. Thus, bacterial isolates from patients with persistent or recurrent infection show extensive adaptive regulatory changes that favour bacterial persistence, including genome reduction and increased antibiotic resistance. Data do not yet support a correlation between phages and acquired pathogenicity in *B. pseudomallei*<sup>97,98</sup>.

## Host immune response

Most patients with melioidosis have at least one predisposing risk factor, suggesting that initiation, progression and outcome of the disease are largely determined by the host's immune status<sup>12,90</sup>. For example, genetic polymorphisms in *TNF* (encoding tumour necrosis factor), *NOD2*, *TLR4* (encoding Toll-like receptor (TLR) 4) and *TLR5* have all been linked to disease severity in patients with melioidosis<sup>74,99–101</sup>. Hypofunctional *TLR5* was associated with decreased organ failure, improved survival and a functional cytokine response, possibly mediated by IL-10 (REF. 102). Interestingly, individuals with the hyporesponsive *TLR5* polymorphism display heightened susceptibility to invasive aspergillosis (diseases caused by infection of fungi of the genus *Aspergillus*) and Legionnaires' disease (atypical pneumonia caused by *Legionella* bacteria)<sup>103</sup>.

## Innate immune response.

*B. pseudomallei* activates the complement pathway, but the bactericidal activity of the complement membrane attack complex is hampered by the external capsular polysaccharides of the bacteria<sup>104</sup>. Owing to its capsule and lipopolysaccharides (LPSs), *B. pseudomallei* is also resistant to lysosomal defensins and cationic peptides (which contribute to bacterial killing by disrupting the structure of the cell membrane of the pathogen), enabling survival in human serum and within phagocytes<sup>44</sup>.

Neutrophil, macrophage and lymphocyte recruitment at the point of infection is triggered by activation of pattern recognition receptors, such as TLRs. Despite the possible detrimental effects of excessive neutrophil recruitment<sup>105</sup>, activated neutrophils play a pivotal part in early bacterial containment<sup>106</sup>.

TLRs recognize conserved pathogen-associated molecular patterns (PAMPs) and mediate an inflammatory immune response via various signalling adaptor proteins, including myeloid differentiation primary response protein MyD88 (FIG. 3). *MyD88* downregulation in experimental melioidosis increases susceptibility to infection as a result of diminished neutrophil recruitment and activation<sup>107</sup>. *B. pseudomallei* triggers the upregulation of multiple TLRs including TLR2, TLR4 (and its co-receptor monocyte differentiation antigen CD14) and TLR5 in host cells, leading to the production of pro-inflammatory and anti-inflammatory cytokines via nuclear factor- $\kappa$ B (NF- $\kappa$ B)<sup>108–110</sup>. TLR signalling can be dampened or dysregulated by structural variants of LPSs<sup>111,112</sup>. In addition, LPS recognition seems to be model-dependent: it occurs solely through TLR4 in murine models, whereas in humans, TLR2 has an additional role<sup>113</sup>.

Phagosomal escape exposes *B. pseudomallei* to intra-cellular TLR-independent pattern recognition receptors, namely, NOD-like receptors, and activates the formation of the inflammasome, a multimeric protein complex that includes a sensor molecule and caspase 1 (REF. 114). Once the sensor molecule detects *B. pseudomallei* PAMPs, caspase 1 is activated and rapid pyroptosis ensues<sup>115</sup> (FIG. 3). Additionally, the activation of caspase 1 releases active IL-1 $\beta$  and IL-18, which are both increased in patients with septic melioidosis<sup>105,116–118</sup>. IL-18 contributes to IFN $\gamma$  induction and, therefore, has a protective effect against *B. pseudomallei* infection<sup>105,116</sup>, whereas IL-1 $\beta$  has a potential deleterious role owing to excessive recruitment of neutrophils. This interplay supports intracellular bacterial growth, tissue damage and inhibition of IFN $\gamma$  production<sup>105</sup>.

### Adaptive immune response.

Although *B. pseudomallei* antibodies (due to either past or asymptomatic infection) are common in individuals from melioidosis-endemic regions, their role in developing functional immunity to melioidosis is ambiguous, as reinfection from different strains is possible and, therefore, can occur even in the presence of high antibody levels<sup>91</sup>.

A strong, comprehensive, cell-mediated immune response is essential for protection against progression of infection and for bacterial clearance<sup>119</sup>. CD4<sup>+</sup> T cells are paramount for B cell isotype switching and for activation of cytotoxic CD8<sup>+</sup> T cells and macrophages<sup>120</sup>. Consistent with this, human survivors of melioidosis display increased levels of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, whereas a decrease in the levels of these cells is specifically correlated with greater mortality. Moreover, vaccines that evoke an immune response skewed towards the activation of T helper 1 (T<sub>H</sub>1) cells (which promote cellular responses) provide protection against melioidosis, with potential to generate sterilizing immunity (which protects from the onset of both disease and infection)<sup>121,122</sup>.

Gradually, granulomas (containing neutrophils, macrophages, lymphocytes and MNGCs) form at the site of infection. Intracellular ‘globes’ of bacteria are seen within MNGCs in a background of acute necrotizing inflammation<sup>123</sup>, which could lead to the development of granulomas. Unfortunately, the dynamic of granuloma formation has not been studied as extensively in melioidosis as in tuberculosis, but it has been recognized as a source of bacterial reactivation in persistent or latent infections. Most clinicians would advocate active

investigation of the nature of granulomas incidentally found in patients from melioidosis-endemic regions.

Unlike in infections caused by other organisms with pathogenicity mechanisms similar to those of *B. pseudomallei* (such as, for example, non-typhoidal *Salmonella*<sup>124</sup>), HIV infection does not seem to be a risk factor for melioidosis or for a more-severe or fatal outcome<sup>125</sup>. In individuals with HIV infection, macrophages show a dysregulated cytokine response (for example, of TNF, IL-10 and IL-12) owing to a low CD4<sup>+</sup> T cell count but retain the capacity for bacterial internalization and intracellular killing<sup>126</sup>. In murine models of melioidosis, depletion of T cells and natural killer cells (hence a 95% reduction in IFN $\gamma$  production) did not hamper bacterial control, suggesting substantial redundancy of defence mechanisms and the involvement of macrophages expressing major histocompatibility complex (MHC) class II<sup>127</sup>. For primary melioidosis, it has been suggested that bystander T cell activation is not required for host survival and could play a more substantial part during the antigen-induced activation phase than during the cytokine-mediated activation<sup>127</sup>. Paradoxically, a strong CD4<sup>+</sup> and CD8<sup>+</sup> T cell response was observed during acute infection in patients with melioidosis, and lower responses were associated with increased mortality<sup>121</sup>. Although the current weight of evidence favours a role for T cells in late stages of infection, little is known about the role of specific T cell subsets in regulating the speed of progression or course of *B. pseudomallei* infection<sup>15</sup>.

### The inflammatory response.

The initial pro-inflammatory response to *B. pseudomallei* infection is a protective, bacterial killing mechanism. However, a dysregulated cytokine-mediated immune response could result in excessive inflammation with a potentially fatal outcome<sup>90</sup>. Elevated levels of pro-inflammatory cytokines (such as IL-6, IL-12, IL-15, IL-18, TNF and IFN $\gamma$ ) have been observed in patients with melioidosis, some of which have been correlated with a fatal outcome (for example, IL-6 and IL-18 are considered mortality predictors)<sup>116,128</sup>. IFN $\gamma$  production activates T cells and natural killer cells; in murine models, natural killer cells are detected at the site of infection and produce 60–80% of the secreted IFN $\gamma$ <sup>106,129</sup>.

Abrogation of TNF or its receptors (in knockout models) results in susceptibility to melioidosis, with increased neutrophil-based inflammatory influx and associated necrosis<sup>106,130,131</sup>. It is postulated that, in the absence of TNF, a hyperproduction of cytokines and chemokines occurs, leading to septic shock and mortality<sup>130</sup>.

Anti-inflammatory cytokines (such as IL-10 and IL-4), TNF receptor type I (TNFR1; also known as TNFRSF1A) and IL-1 receptor antagonist (IL1RA) are also upregulated during septic melioidosis. Significant increases in IL-1RA and TNFR1 expression were observed in non-survivors<sup>117,132</sup>. Fate-onset inflammatory mediators are also correlated with clinical outcome and mortality<sup>133,134</sup>: in experimental melioidosis, macrophage migration inhibitory factor (MIF) impairs bacterial defence, and neutralizing antibodies against high mobility group protein B1 (HMGB1) could be used as an adjunctive therapy to improve outcome. Coagulation also has a role in melioidosis severity (BOX 4).

## Diagnosis, screening and prevention

Melioidosis is grossly underdiagnosed worldwide<sup>3</sup>, mainly owing to a lack of diagnostic microbiological laboratories serving the low-income rural populations who are at greatest risk of infection and a lack of awareness of the disease amongst physicians and laboratory staff. Even in good microbiological laboratories, *B. pseudomallei* could initially be discarded as a contaminant (a commensal or non-pathogenic environmental species), especially in non-endemic areas<sup>135</sup>.

### Clinical diagnosis

The incubation period of acute infections is on average 9 days<sup>136</sup>, ranging from 1–21 days, although a more severe form of the disease with shorter incubation can occur after inhalation or aspiration of contaminated fresh water<sup>137</sup>. In patients with melioidosis, the clinical presentation, severity and outcome are affected by the presence or absence of risk factors, the route of infection and the bacterial load and strain, as well as the presence or absence of specific non-ubiquitous *B. pseudomallei* virulence genes<sup>12,83</sup>. The clinical spectrum of disease varies from localized cutaneous manifestations at the bacterial entry site with no systemic manifestations to sepsis and death (FIG. 4). Bacteraemia on admission occurs in 40–60% of all patients diagnosed with melioidosis, and septic shock occurs in ~20% of all cases. Pneumonia is the presenting illness in about half of all cases. Dissemination of the bacteria to internal organs is common, especially the spleen, prostate, liver and kidneys.

**Limitations of clinical diagnosis.**—Making a diagnosis on clinical grounds alone is very difficult, although in known endemic areas, a patient with suggestive clinical and epidemiological (the presence of risk factors, such as diabetes mellitus and occupational or seasonal exposure) features should be treated empirically with antibiotics that target *B. pseudomallei*. As the clinical presentation of melioidosis can be nonspecific, the disease should be considered in anyone with a fever in endemic and potentially endemic countries (listed in REF. 3), in particular in individuals with abscesses (especially in the liver, spleen, prostate or parotid) or pneumonia. However, not all patients have risk factors. Laboratory tests are required to confirm the diagnosis of melioidosis<sup>138</sup>.

### Microbiological diagnosis

**Culture.**—Culture remains the mainstay of melioidosis diagnosis. *B. pseudomallei* can grow on most routine laboratory media but might not be recognized and could be dismissed as a contaminant, or could be mis-identified as other bacteria (such as *Pseudomonas* spp. and *Bacillus* spp.) unless laboratory staff are familiar with its appearance<sup>138</sup> (FIG. 5). *B. pseudomallei* is never part of the normal human flora, and its isolation from any clinical sample should be regarded as diagnostic of melioidosis. As *B. pseudomallei* is classified as a hazard group 3 pathogen and tier 1 select agent<sup>139</sup>, doctors should alert the hospital laboratory if melioidosis is suspected in an admitted patient to ensure that appropriate precautions can be followed. All microbiology laboratory staff in melioidosis-endemic areas should receive appropriate training regularly and follow local safety standards.

It is crucial that the appropriate clinical samples are collected and sent for culture to laboratories that are familiar with the disease and its causative organism. Blood cultures are the most important sample, as bacteraemia is common. Culture of throat or rectal swabs<sup>140,141</sup> and the centrifuged deposit from urine<sup>142</sup> are also recommended in all cases of suspected melioidosis, as these could be the only positive samples in some patients. Other samples that should be cultured include pus from abscesses and sputum in patients with pneumonia. Although blood cultures are positive in ~50% of patients overall<sup>7</sup> and in up to 75% of patients in some reports<sup>143,144</sup>, this proportion is lower in children<sup>38</sup>, potentially reflecting that they usually do not have classic melioidosis risk factors. Because cultures have low sensitivity (60%)<sup>145</sup>, repeating cultures (especially of blood, sputum, urine and pus samples) should be considered in patients with strong indications of *B. pseudomallei* infection, as it is not uncommon to find subsequent samples positive despite initial negative results. If patients do not improve after 3–7 days of treatment for melioidosis and all culture results are negative, further investigations and a re-evaluation of the diagnosis and treatment should be considered.

*B. pseudomallei* grows on most routine laboratory media, but more slowly than many other organisms and, therefore, it might be outgrown in samples from sites that normally have a microbial flora; thus, selective media are preferable. Agar plates should be incubated and inspected daily for up to 4 days in suspected cases.

Standard biochemical tests and kit-based identification methods can be used to confirm the identity of colonies. As misidentification of *B. pseudomallei* with such methods are not uncommon<sup>138</sup>, the use of multiple methods is recommended. An antibody–antigen binding approach using a monoclonal antibody-based latex agglutination assay is very useful for screening suspect colonies<sup>146</sup>. Increasing numbers of clinical laboratories use matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry for bacterial identification, as this method (combined with appropriate databases) can provide an accurate identification of *B. pseudomallei*<sup>147</sup>. Numerous molecular approaches for species identification have been described, including 16S rDNA sequencing<sup>148</sup> and specific PCRs<sup>149</sup>, although these tests 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 disc diffusion antibiotic sensitivity test (to determine the resistance to gentamicin and colistin (or polymyxin) and the susceptibility to amoxicillin-clavulanic acid (also known as co-amoxiclav)) has been recommended to screen Gram-negative rod-shaped bacteria that produce cytochrome oxidase<sup>138,143</sup>, although it should be noted that gentamicin-susceptible isolates of *B. pseudomallei* predominate in some regions<sup>144</sup>.

**Direct detection in clinical samples.**—Because melioidosis can have severe, if not fatal, consequences, treatment should not be delayed by waiting days for culture results; thus, direct detection of the organism in clinical samples could provide a quick confirmation of the diagnosis. *B. pseudomallei* can be observed with light microscopy (the organism is often described in textbooks as a Gram-negative rod-shaped bacterium with bipolar staining that resembles a safety pin), but light microscopy lacks sensitivity and specificity<sup>150</sup>.

Immunofluorescent microscopy has a specificity approaching 100%, although the sensitivity is <50% compared with culture<sup>151</sup>.

Alternative approaches using antigen detection or nucleic acid amplification have also been used. A lateral flow immunoassay that detects the extracellular capsular polysaccharides has been developed<sup>152</sup>, but it has not yet been extensively evaluated and, although it shows good specificity, it seems to have poor sensitivity, especially for blood specimens<sup>153</sup>. Numerous PCR assays with high specificity for *B. pseudomallei* have been developed since the 1990s and have undergone small-scale clinical evaluations. The most promising assay targets the T3SS gene cluster<sup>154</sup>, although the sensitivity in blood samples depends at least in part on an adequate bacterial concentration<sup>155</sup>. However, these PCR assays are not routinely used for clinical diagnosis in endemic areas, even in high-income countries such as Singapore and Australia: in addition to sensitivity issues, these tests are not cost-effective in providing the timely confirmation of diagnosis, which clinicians need to make therapeutic decisions.

**Serology.**—The serological diagnosis of melioidosis is difficult. Many different assays have been developed for detecting antibodies against *B. pseudomallei*, but most of them are based on poorly characterized antigens and have never been internationally standardized or subjected to extensive critical evaluation. The most widely used is an indirect haemagglutination test (a simple serological test used to detect antibodies raised against *B. pseudomallei*). The background seropositivity rates in the healthy population in some endemic areas are very high, presumably because of repeated exposures to *B. pseudomallei* or closely related organisms<sup>156,157</sup>. As a result, many patients presenting with fever are misdiagnosed with melioidosis in endemic countries in southeast Asia on the basis of a positive indirect haemagglutination test. By contrast, some patients with melioidosis never mount a good antibody response, perhaps because their immune system is compromised. The indirect haemagglutination test on admission has a reported sensitivity of only 56% in Australia<sup>158</sup> and 73% in Thailand<sup>157</sup>, although in the Australian study, 68% of the patients whose tests were negative on admission subsequently showed seroconversion<sup>158</sup>. Thus, the diagnosis of melioidosis should not rely on the indirect haemagglutination test.

New assays based on purified antigens are being developed and have undergone small-scale evaluations, with some evidence of improved sensitivity and specificity<sup>159–161</sup>. A protein microarray that contains 20 recombinant and purified *B. pseudomallei* proteins provides a standardized, easy-to-perform test for the detection of *B. pseudomallei*-specific antibody patterns<sup>162</sup> and could have the potential to improve the serodiagnosis of melioidosis in clinical settings.

## Prevention

In northern Australia, basic public health advice is given every year to the general population, and especially to high-risk groups, such as avoiding direct contact with soil and water at the start of each rainy season<sup>163</sup>. 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, only drink bottled or boiled water and avoid outdoor exposure to heavy rain or dust clouds<sup>5</sup>.

The guidelines also encourage cessation of smoking (particularly in those with underlying conditions such as diabetes mellitus) and discourage the application of herbal remedies or organic substances to wounds<sup>5</sup>. However, the effectiveness of this advice in reducing the incidence of infection has not been proven. Large-scale water chlorination has been very successful in Australia despite theoretical concerns about *B. pseudomallei* survival in some conditions<sup>164</sup>. In low-income and middle-income countries, water should be boiled before consumption. Ultraviolet light treatment is effective for remediation of water contaminated with *B. pseudomallei* and could be recommended in high-income countries in households where individuals are at increased risk of contracting melioidosis<sup>165</sup>.

However, public awareness of melioidosis in developing tropical countries is limited, and preventive approaches are not always adopted<sup>166</sup>. A multifaceted intervention at community and government levels is required for successful prevention and is currently being prospectively evaluated in northeast Thailand<sup>167</sup>.

If high-risk laboratory exposure to *B. pseudomallei* occurs, post-exposure prophylaxis (PEP) is recommended; high-risk incidents include the exposure of penetrating injuries, mouth or eyes to *B. pseudomallei*-contaminated materials and the generation of aerosols outside of a biological safety cabinet<sup>168</sup>. PEP consists of oral antimicrobial treatment with trimethoprim–sulfamethoxazole or, if the organism is resistant or the patient is intolerant, doxycycline or co-amoxiclav for 21 days<sup>168</sup>. The potential benefit of PEP must be weighed against the fact that trimethoprim–sulfamethoxazole can have severe adverse effects: for individuals involved in a low-risk incident, the decision to begin PEP should be based on the presence of known risk factors for naturally acquired melioidosis. Individuals with known risk factors should be advised to receive PEP, whereas in the absence of known risk factors monitoring is sufficient<sup>143,168</sup>.

## Management

Early diagnosis and the start of antimicrobial therapy specific to *B. pseudomallei* are crucial for melioidosis treatment. 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 ~10%<sup>12</sup>. Nevertheless, such resources are not available or are limited in many endemic regions, and in those circumstances, mortality is 40%<sup>12</sup>. The majority of *B. pseudomallei* isolates from primary infections have the same characteristic antimicrobial susceptibility profiles. *B. pseudomallei* is susceptible to  $\beta$ -lactam antibiotics (such as ceftazidime, meropenem, imipenem and co-amoxiclav), although the bactericidal activity of these drugs varies, and is almost always susceptible to doxycycline, chloramphenicol and trimethoprim–sulfamethoxazole<sup>169–171</sup>, although these agents only have bacteriostatic activity. Most isolates are susceptible *in vitro* to piperacillin, ceftriaxone and cefotaxime, but these agents are less effective clinically<sup>172</sup>. However, *B. pseudomallei* is resistant to penicillin, ampicillin, first-generation and second-generation cephalosporins, gentamicin, tobramycin, streptomycin, macrolides and polymyxins (BOX 3). Of note, clonal groups of isolates susceptible to gentamicin are common in Sarawak, Malaysia<sup>144</sup>.

On the back of the global concerns of antimicrobial resistance and the already limited options for treating melioidosis, new antimicrobials have been tested *in vitro* and in animal models, but none can yet replace ceftazidime and meropenem<sup>173</sup>. Doripenem has minimum inhibitory concentrations (the lowest concentrations that can prevent visible bacterial growth after over-night incubation) similar to meropenem, but ertapenem, tigecycline and moxifloxacin seem to have limited *in vitro* activity<sup>174</sup>.

As melioidosis is not a contagious disease, isolation of patients or special precautions are usually not required within endemic areas. However, as few nosocomial infections have been reported<sup>24,175,176</sup>, healthcare providers are recommended to follow universal precautions<sup>177</sup> and standard infection control practices, including hand hygiene<sup>178</sup>. Potential contamination of the ward and local environment from patients with superficial lesions or pneumonia has been raised as a concern, but such contamination has never been documented.

Formal guidelines for melioidosis therapy, including recommended dosage and duration of each therapeutic phase, have been published by the CDC after a 2010 expert workshop that updated prior consensus-based guidelines<sup>168</sup>. Antimicrobial therapy consists of the initial intensive phase and the subsequent eradication phase (BOX 5).

### Initial intensive therapy

Intravenous ceftazidime or meropenem is the preferred choice<sup>179</sup>; the duration of initial intensive therapy should last a minimum of 10–14 days, with longer intensive therapy for critically ill patients, including those with extensive pulmonary disease, deep-seated collections or organ abscesses, osteomyelitis (infection of bone), septic arthritis or neurological melioidosis (BOX 5). The therapeutic response can be slow; the median time to resolution of fever is up to 9 days<sup>180</sup>, with longer times in patients with deep-seated abscesses. The addition of trimethoprim–sulfamethoxazole to ceftazidime for the intensive phase is used by some physicians for certain types of infection (BOX 5) but conferred no survival benefit in studies in Thailand<sup>181,182</sup>.

Imipenem and meropenem have the lowest minimum inhibitory concentrations against *B. pseudomallei* and have faster bacterial killing rates than ceftazidime *in vitro*<sup>183,184</sup>. The recommendation of meropenem as the drug of choice for severe melioidosis with septic shock is also supported by observational data from Australia<sup>185</sup>. However, ceftazidime remains the drug of choice for initial therapy for most patients with melioidosis, and there is no conclusive evidence that meropenem is superior to ceftazidime in patients who are not critically ill. In Australia, meropenem is switched to ceftazidime when those patients with severe disease recover and are well enough to be discharged from the intensive care unit to general wards.

### Eradication therapy

After the initial intensive therapy, subsequent eradication therapy with oral antibiotics is recommended to prevent recrudescence of the disease or relapse of the patient. Trimethoprim–sulfamethoxazole is the preferred agent for eradication therapy (BOX 5), and co-amoxiclav or doxycycline is the second choice. Reports of primary resistance to

trimethoprim–sulfamethoxazole in >10% of *B. pseudomallei* isolates from Thailand and other southeast Asian countries were demonstrated to be inaccurate<sup>171</sup>.

Based on empirical experience and therapeutic modelling, dosing recommendations for trimethoprim–sulfamethoxazole<sup>186</sup> and co-amoxiclav<sup>187</sup> in melioidosis are higher than the standard doses used with these antibiotics. Consequently, owing to the long-term course of trimethoprim–sulfamethoxazole required, adverse effects are reported in up to 40% of patients<sup>10</sup>. In Thailand, this drug is usually avoided in patients who have glucose-6-phosphate dehydrogenase (G6PD) deficiency, owing to the risk of haemolytic anaemia, although patients are not routinely screened for G6PD activity prior to treatment. Rash, gastrointestinal symptoms, hyperkalaemia (high serum potassium levels that can lead to muscle weakness and arrhythmias) and rising levels of serum creatinine (which could indicate renal dysfunction) could require dose modification or cessation of trimethoprim–sulfamethoxazole therapy, which can be replaced by doxycycline or co-amoxiclav. Desensitization (a strategy to safely induce drug tolerance and limit the possibility of a type I hypersensitivity reaction) should be considered for non-severe skin reactions attributed to trimethoprim–sulfamethoxazole.

In Australia, trimethoprim–sulfamethoxazole is the preferred eradication therapy for children and potentially in pregnant women after the first trimester (owing to the risk of neural tube or other congenital defects), whereas in some locations in Thailand co-amoxiclav has been used for eradication therapy in children and in pregnancy. However, dosing with co-amoxiclav is problematic, and acquired resistance is well documented when co-amoxiclav or doxycycline is used<sup>4</sup>.

### Treatment duration

Lengthening the duration of the initial intensive therapy for patients with more-severe melioidosis has contributed to the decrease in mortality in regions with the necessary healthcare resources. A retrospective analysis of patients who were treated according to the Royal Darwin Hospital melioidosis treatment guidelines, which define the minimum recommended duration of initial intensive therapy based on the clinical presentation<sup>188</sup>, supports a longer intravenous therapy for critically ill patients. The median duration of the initial intensive therapy for such patients in that analysis was ~4 weeks and only 5 patients (1.2%) relapsed. Although non-compliance of patients during eradication therapy is common as patients stop taking the antibiotics early, skip doses or do not take any drug at all after discharge, the relapse rates in the Darwin study are low and are attributed to prolongation of the initial intensive therapy<sup>188</sup>. Studies are required to assess whether future guidelines can include options based solely on intravenous therapy without a long-term eradication phase.

Studies from Thailand showed that failure of the eradication therapy is associated with poor adherence to therapy, more-severe infections (for example, multifocal disease and bacteraemia) and a duration of eradication therapy of <8 weeks<sup>189–191</sup>. These findings support the recommendations for an eradication therapy of 3–6 months (BOX 5). Case series reported that selected patients with localized cutaneous disease could be successfully treated with oral trimethoprim–sulfamethoxazole for 3 months without preceding initial intensive

therapy<sup>34,192,193</sup>. However, such a regimen must be restricted to patients who have no signs of sepsis or organ dysfunction, no underlying risk factors and no dissemination of the infection to distant sites, including regional lymph nodes.

### Surgical aspects of therapy

Surgical drainage is usually required for single, large abscesses in the liver and muscles and prostatic abscesses<sup>194</sup>, but it is not necessary or possible for multiple small abscesses in the spleen, liver and kidneys. Septic arthritis (inflammation of the joints due to the infection) usually requires drainage and washout and might require repeated procedures. Other internal abscesses rarely need to be drained as they frequently resolve with medical therapy. Osteomyelitis can be very extensive when diagnosis and appropriate antibiotic therapy are delayed, and in such cases, aggressive and often repeated surgical debridement of the necrotic bone is usually necessary<sup>195</sup>. However, early long-bone osteomyelitis without abscess formation and vertebral osteomyelitis without epidural abscess might not require debridement. Mycotic aneurysms (caused by bacterial infiltration of the arterial wall) require urgent surgery, often with insertion of prosthetic vascular grafts. Lifelong suppressive therapy with trimethoprim–sulfamethoxazole might be indicated for patients who have received prosthetic grafts for mycotic aneurysms.

### Adjuvant therapy

State-of-the-art intensive care management can substantially decrease mortality in patients with melioidosis and sepsis or septic shock<sup>196,197</sup>, and appropriate guidelines are available<sup>198</sup>.

Granulocyte colony-stimulating factor (G-CSF, which stimulates the production of neutrophils in the bone marrow) has been used empirically in patients with septic shock. The rationale is to counteract the functional neutrophil defects that are thought to be crucial in the pathogenesis of severe melioidosis. Early observational data showed a significant improvement in survival with G-CSF, but this result was confounded by concomitant improvements in other clinical parameters<sup>199</sup>. A subsequent randomized controlled trial in Thailand showed no overall survival benefit with addition of G-CSF<sup>200</sup>; nevertheless, survival was significantly longer in the G-CSF group in settings with limited intensive care resources, and adjuvant G-CSF is still used for melioidosis-associated septic shock in some hospitals with state-of-the-art intensive care facilities. Preclinical studies have shown that administration of clinically available IL-1 $\beta$  blocking agents can protect mice against overt *B. pseudomallei* infection and mortality<sup>105,201</sup>. This finding is in line with reports that patients with melioidosis who have diabetes mellitus and take glibenclamide (also known as glyburide) have a lower mortality and attenuated inflammatory responses compared with patients who do not take glibenclamide<sup>202</sup> (BOX 1). Given the crucial role of immune function in melioidosis pathogenesis, patients with melioidosis-associated sepsis or septic shock could benefit from newly available immune-modulating therapies, such as IL-7, granulocyte-macrophage colony-stimulating factor and anti-PD1 (programmed cell death protein 1).

## Quality of life

### Recrudescence and recurrence of melioidosis

Recrudescence of melioidosis with a return of clinical illness and culture positivity can occur during the eradication treatment period if the initial intensive therapy is ceased too early, internal abscesses have not been diagnosed or adequately drained or if the oral eradication therapy is not adhered to or is ceased too early. Patients with recrudescence will need hospital admission, intravenous parenteral antimicrobials and re-examination for sites of infection.

Once both initial intensive and eradication therapies have been successfully completed, recurrent melioidosis could be due to either recrudescence of the original infection, as confirmed by bacterial isolate genotyping, or a new infection with a different strain<sup>190,203</sup>. With improvements in therapy, recrudescence decreased from ~10% of cases to <5%; new infections in survivors of melioidosis are now more common than recrudescence<sup>188</sup>.

### Long-term sequelae

For survivors of melioidosis, the main determinants of future health are their underlying risk factors that predisposed them to the initial infection. Many of the patients in the Darwin Prospective Melioidosis Study have subsequently died or had a substantial and ongoing disability as a consequence of comorbidities such as diabetes mellitus, chronic kidney disease or malignancy (B.J.C., unpublished observation). The most severe melioidosis-associated disability is residual neurological deficit subsequent to melioidosis-associated encephalomyelitis. Although rare, neurological complications are particularly problematic in children and can range from severe residual quadriparesis (muscle weakness in all limbs) or severe flaccid paraparesis (partial or complete paralysis of both legs) to persisting isolated foot drop (an abnormal gait in which the forefoot drops owing to weakness)<sup>34</sup>. Limited range of motion, sinus tract (an infected tract connecting a deep-seated infection to the surface or another organ and often discharging pus) formation and joint deformities are also common in patients with bone and joint infections<sup>195,204</sup>.

## Outlook

### Melioidosis as a neglected disease

Neglected tropical diseases are understudied diseases that remain endemic in many developing countries around the world<sup>205</sup>. Melioidosis is not included in most lists of neglected tropical diseases, including the WHO list<sup>205</sup>, even though it has high mortality and is potentially preventable and treatable<sup>3</sup>. Thus, efforts by the international research community are needed to raise awareness of melioidosis within the WHO and regional and local health agencies, as well as in the general public in endemic areas. 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 \(http://www.melioidosis.info\)](http://www.melioidosis.info).

## Melioidosis in developing countries

**Diagnosis.**—In developing countries, melioidosis is most common amongst members of the rural population, who often have limited access to even simple diagnostics. Furthermore, even if clinical microbiology laboratories are available, they might be underused owing to costs or lack of trained staff<sup>206</sup>. Non-culture-based methods to diagnose bacterial infectious diseases are increasingly being encouraged<sup>207</sup>, but they have not been extensively developed and evaluated for melioidosis. Thus, the priorities for improving melioidosis case ascertainment in tropical endemic areas are the establishment of basic clinical microbiology laboratories and education of both clinicians and laboratory technicians about melioidosis and prevention.

**Management.**—To reduce acute melioidosis mortality, the availability and affordability of ceftazidime or carbapenems need to improve. In Thailand, an upper-middle-income country, a 14-day course of ceftazidime or carbapenem costs ~US\$60 or US\$1,080, respectively, and is covered by Thailand's Universal Coverage Scheme<sup>208</sup>. Nonetheless, in other low-income and middle-income countries, these drugs could be more expensive, have limited availability or be excluded from the country's universal health coverage, and patients frequently cannot afford to pay for the drugs themselves<sup>200</sup>. Studies on how best to allocate and use resources for melioidosis in low-income and middle-income countries are urgently needed.

## The One Health Initiative

In 2016, melioidosis was highlighted as a zoonosis, a disease of animals and humans caused by an environmental organism; however, the disease in animals and geographical distribution of distinct human clinical manifestations (for example, parotitis is mainly observed in children within southeast Asia and encephalomyelitis mainly in northern Australia) are not well understood<sup>210</sup>. Thus, adopting the One Health Initiative (a strategy involving interdisciplinary collaborations of health professionals at the local, national and global levels in all aspects of healthcare for humans, animals and the environment<sup>211</sup>) will promote cooperation and strategic planning between physicians, ecologists, environmental scientists and veterinarians. Furthermore, interdisciplinary efforts will help to address the zoonotic spread of the disease and to establish effective melioidosis interventions.

To implement One Health Initiative approaches, potential technology-based solutions, such as wireless and mobile technologies for the delivery of health interventions and education and the interactive tools currently displayed on the aforementioned melioidosis website (<http://www.melioidosis.info>), should be implemented in resource-limited settings. One Health Initiatives for melioidosis could also lead to a broader engagement of organizations and individuals with experience in prevention, surveillance and clinical case management of neglected tropical diseases, as well as individuals with training in economic development, genomics, veterinary sciences, wildlife management, agriculture, molecular biology and bacteriology, ecology, policy and law<sup>211</sup>. The implementation of this interdisciplinary initiative would combine field efforts addressing both endemic and emerging melioidosis but requires effective global health governance<sup>210</sup>.

## Advances in vaccine development

A vaccine could be a cost-effective intervention in tropical developing countries, particularly if used in high-risk populations such as individuals with diabetes mellitus, even if it produces only partial immunity<sup>212</sup>. The Steering Group on Melioidosis Vaccine Development has highlighted the need for standardized animal models and for advancing melioidosis vaccine candidates to preclinical and clinical studies, and the challenges posed by bacterial strains and routes of immunization. Furthermore, it recognized that vaccines designed for the military or other populations involved in biodefense have different requirements from those designed to prevent naturally acquired melioidosis in endemic areas<sup>213</sup>.

No melioidosis vaccine is currently available for human use; some vaccine candidates have been shown to provide partial protection against melioidosis or glanders in murine models of infection<sup>212,214,215</sup>, but few have been tested in non-human primates or humans<sup>216</sup>. Live attenuated vaccines induce a more-comprehensive immune response in animal models and are currently considered the best approach for generating protection against *B. pseudomallei* infection<sup>16,217</sup>. However, subunit-based vaccines provide a feasible alternative because of their increased safety and potential for large-scale production. Experimental evidence indicates that the combination of bacterial polysaccharides (LPSs or other capsular polysaccharides) with well-defined protein antigens (glycoconjugates) can generate substantial protection against *Burkholderia* infections<sup>217</sup>. This rapid advancement in vaccine design and optimization is very promising, and several vaccine candidates are currently being tested. However, a multivalent vaccine containing numerous immunogenic bacterial components will probably be necessary to achieve complete protection, as, in addition to a strong antibody response, both CD4<sup>+</sup> and CD8<sup>+</sup> T cells are also important in protection against human melioidosis.

## Final thoughts

New global environmental sampling studies and improvements in diagnostic microbiology could enhance the understanding of the geographical distribution and burden of *B. pseudomallei*, and wide-scale whole-genome sequencing together with clinical details could provide new insights into the phylogeny and virulence of these bacteria. The application of such new techniques to isolates with well-characterized clinical and epidemiological metadata could provide further insights into melioidosis. New omics-based technologies will enable a better understanding of how *B. pseudomallei* evades immune surveillance and can remain latent for many years. The role of the human and environmental microbiota is only just beginning to emerge<sup>218</sup> and could offer creative insights into how to tackle the infection *in vivo* and reduce exposure *in terra*.

Affordable, effective alternative drugs and new drug targets are needed to reduce mortality, relapse and the duration of treatment courses. More than 80% of individuals with diabetes mellitus live in low-income and middle-income countries, and the numbers are projected to increase by >55% globally by 2050, with tropical countries facing the brunt of this epidemic<sup>219</sup>. Case clusters of melioidosis have also been associated with severe weather events<sup>220,221</sup>, which, based on the current estimates of global climate change, might become

increasingly common. This combination could cause a surge of melioidosis, particularly in countries where it has previously been under-reported, such as India. Calculating a representative disability-adjusted life year metric could also provide better comparative burden information and prompt regulating bodies to recognize melioidosis as a neglected tropical disease.

## Acknowledgements

W.J.W. received a Vidi grant (91716475) from the Netherlands Organisation for Scientific Research (NWO) and Marie Curie Skledowska Innovative Training Network (MC-ITN) European Sepsis Academy, funded by the European Union's Horizon 2020 programme. H.S.V. received a Marie Curie Skledowska fellowship under the European Sepsis Academy, funded by the European Union's Horizon 2020 programme. A.G.T. is supported by the NIH and the National Institute of Allergy and Infectious Diseases (NIAID) R01 grant AI12660101. B.J.C. is supported by Australian National Health and Medical Research Council grants, including the HOT NORTH initiative. S.J.P. is an NIH Research Senior Investigator. D.A.B.D. is supported by The Wellcome Trust of Great Britain (grant number 106698/Z/14/Z). D.L. is supported by The Wellcome Trust Public Health and Tropical Medicine Intermediate Fellowship (grant number 101103/Z/13/Z). The authors thank G. Wongsuvan, P. Amornchai, P. Wannapinij and V. Wuthiekanun for their assistance with the images in FIG. 5.

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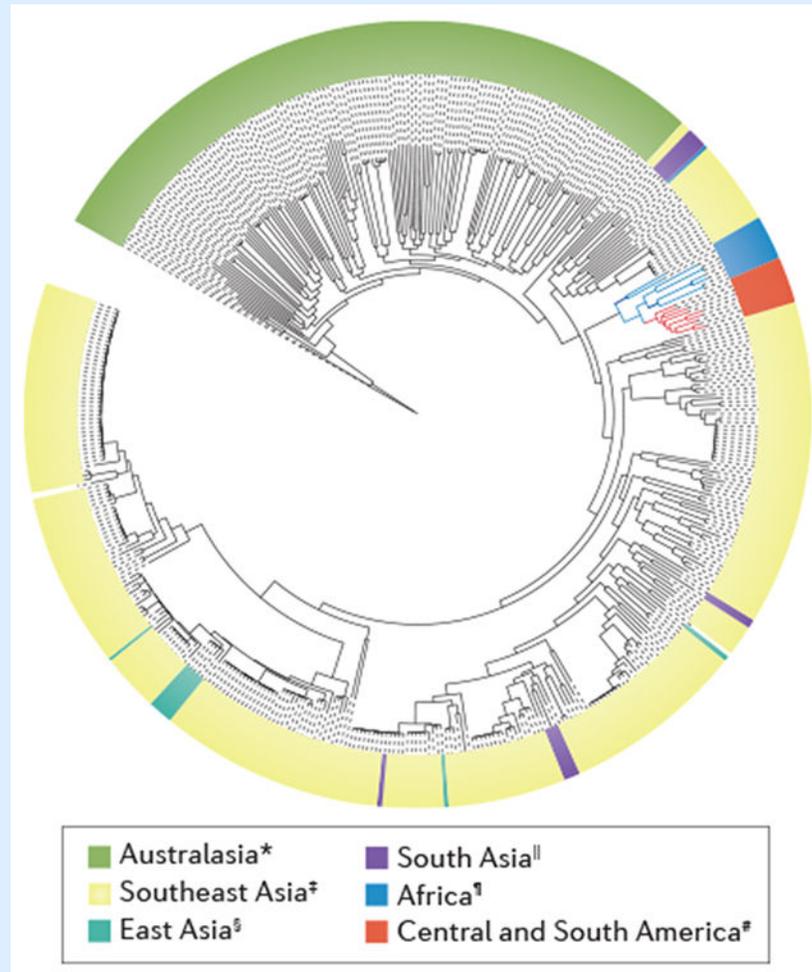
**Box 1 |****Diabetes mellitus and melioidosis**

There is a strong correlation between diabetes mellitus and *Burkholderia pseudomallei* infection, as 23–60% of patients with melioidosis also have diabetes<sup>12</sup>. Diabetes results in blunted *B. pseudomallei*-specific cellular responses during acute infection<sup>121</sup>, including decreased capacity for macrophages to phagocytose and kill the bacteria, reduced lipopolysaccharide-induced generation of CD4<sup>+</sup> regulatory T (T<sub>reg</sub>) cells and impairment of Toll-like receptor-mediated myeloid differentiation primary response protein MyD88 inflammatory signalling. Dysregulated phosphorylation of nuclear factor- $\kappa$ B results in excessive tumour necrosis factor and IL-12 production by mononuclear cells, resulting in greater risk of septic shock<sup>222,223</sup>. Furthermore, disease progression and severity in diabetes is exacerbated by loss of effective proliferation of CD4<sup>+</sup> T cells (which express higher levels of cytotoxic T lymphocyte protein 4) and loss of CD4<sup>+</sup> T cell function, which is exacerbated by increased expression of programmed cell death 1 ligand 1 (a known regulator of T cell activation) on neutrophils; these neutrophils also inhibit interferon- $\gamma$  production<sup>224</sup>.

In individuals with diabetes, several studies have demonstrated defects in neutrophil adhesion, chemotaxis and intracellular killing, but studies on the efficiency of neutrophil phagocytosis show mixed results<sup>225</sup>. Conflicting observations could be due to methodological differences; reduced phagocytosis could be explained by decreased opsonization of bacteria (a prerequisite for neutrophil uptake), possibly due to glucose affecting the thioester bond of complement C3 and thereby preventing binding to the bacterial surface<sup>225</sup>. Humoral responses are also poorer and could affect vaccination<sup>225</sup>.

However, diabetes was associated with a lower overall mortality in patients with melioidosis in Thailand, although only in those who were being treated with glibenclamide<sup>202</sup>. Glibenclamide is an anti-inflammatory agent that inhibits IL-1 $\beta$  secretion by monocytes and reduces neutrophil pro-inflammatory cytokine production by lowering free glutathione and enhancing IL-1 receptor-associated kinase 3 (IRAK3) pathways; this results in reduced IL-1 $\beta$  secretion in a dose-dependent fashion<sup>226,227</sup>. Patients taking glibenclamide prior to admission have attenuated inflammatory responses<sup>202</sup>.

## Box 2 |

Genome and phylogeny of *Burkholderia pseudomallei*

The genome of *Burkholderia pseudomallei* consists of two circular replicons — chromosome 1 (4.07Mb) and chromosome 2 (3.17 Mb). Chromosome 1 largely encodes proteins involved in core housekeeping functions, such as cell wall synthesis, metabolism and motility, whereas chromosome 2 mostly encodes proteins required for accessory functions involved in adaptation to environmental conditions<sup>228</sup>. Within this bipartite structure, horizontal gene transfer (transmission of genetic material other than by vertical transmission from parent to offspring) provides genetic plasticity, as represented by the large metabolic repertoire and intrinsic redundancy of virulence factors, such as type III secretion systems<sup>229</sup>. The pan-genome of *B. pseudomallei* shows substantial genetic heterogeneity between strains, which is largely influenced by horizontal gene transfer, recombination and mutations<sup>230,231</sup>.

This highly plastic and, as a consequence, highly variable genome across *B. pseudomallei* strains could also have a role in the various manifestations and disease courses of melioidosis<sup>98,229</sup>. Bacterial genetic mutations can also occur during the infection. For

example, mutations in variable number tandem repeats were detected in isolates collected 2 weeks apart from a patient with acute melioidosis<sup>229,230</sup>. Geographical segregation could also contribute to different clinical manifestations, as region-specific genetic loci are associated with variability in survival and virulence<sup>232</sup>. Whole-genome tiling array expression data demonstrated that non-coding RNA could play an important part in virulence and host-pathogen interactions<sup>40</sup>.

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<sup>232</sup>. Within the endemic zone of southeast Asia, the Mekong subregion has emerged as a hot spot for *B. pseudomallei* evolution<sup>232</sup>. Furthermore, isolates from Africa and Central and South America seem to have a common origin, as suggested by close ancestry that originated between the 17th and 19th centuries<sup>232</sup>.

\*New Caledonia, Australia, Fiji and Papua New Guinea; †Brunei, Cambodia, Indonesia, Lao People's Democratic Republic, Malaysia, Philippines, Singapore, Thailand and Vietnam; §China; ‡India and Bangladesh; ¶Burkina Faso, Chad, Gabon, Kenya, Madagascar, Mauritius and Nigeria; #Ecuador, Brazil, Martinique, Puerto Rico, Venezuela and the Virgin Islands. Figure adapted from (REF. 232), Macmillan Publishers Limited.

**Box 3 |****Antimicrobial drug resistance**

The *Burkholderia pseudomallei* genome contains several genes encoding Ambler class A, B and D  $\beta$ -lactamases. The most important is *penA* on chromosome 2, which encodes a class A membrane-bound lipoprotein that is secreted by the twin-arginine transport system with the ability to hydrolyse most  $\beta$ -lactams<sup>6,233</sup>.

Acquired antimicrobial resistance during melioidosis treatment is rare. Studies of acquired  $\beta$ -lactam resistance (including to carbapenems) whilst on therapy identified three distinct phenotypic changes, mainly resulting from *penA* mutations: derepression of the chromosomal enzyme, insensitivity to  $\beta$ -lactamase inhibitors and specific ceftazidime resistance<sup>6,234</sup>. In isolates from patients in Thailand who did not respond to ceftazidime, large segments of chromosome 2 were deleted, including three genes encoding putative penicillin-binding proteins, which are known targets of  $\beta$ -lactam antibiotics<sup>235</sup>.

Additionally, *omp38*, which encodes an outer membrane porin, is thought to contribute to ceftazidime and carbapenem resistance<sup>233</sup>. Metallo  $\beta$ -lactamase type 2 (encoded by *blaNDM-1*) is a lipoprotein carbapenemase expressed on the outer membrane of Gram-negative bacilli and can be shed in outer membrane vesicles, thereby representing a new mechanism of resistance dissemination that can confer phenotypic resistance to beneficiary bacteria<sup>236</sup>. *B. pseudomallei* *penA* could have a similar purpose<sup>236</sup>. Of note, 2017 reports from isogenic *B. pseudomallei* strains (isolated from the same patients at different time points and traced to a single ancestor) from patients on meropenem have shown increasing minimum inhibitory concentrations<sup>237</sup>.

*B. pseudomallei* lipopolysaccharide structure also plays an intrinsic part in resistance to cationic peptides, such as polymyxin B. *B. pseudomallei* encodes at least ten resistance nodulation division efflux pump systems, spanning both chromosomes, that confer at least partial resistance to six antibiotic classes, including aminoglycosides, fluoroquinolones and tetracyclines<sup>233</sup>. Mutations targeting *folA*, which encodes dihydrofolate reductase, and BpeEF-OprC efflux pump expression confer resistance to trimethoprim<sup>238</sup>. *In vitro*, *B. pseudomallei* cells growing as a biofilm were viable after 24 h of antibiotic (trimethoprim or ceftazidime) exposure, with a minimum inhibitory concentration of up to 200-times that of planktonic bacteria<sup>6</sup>. Inhibition of efflux pumps might lower resistance to ceftazidime and doxycycline in these biofilms<sup>239</sup>.

**Box 4 |****Role of coagulation in melioidosis**

New insights have enhanced our knowledge of the roles of coagulation and fibrinolysis and their interplay with inflammation in the pathogenesis of melioidosis (reviewed in REF. 240). There seems to be a bidirectional role of inflammation and coagulation during melioidosis: activation of coagulation and subsequent fibrin deposition plays an essential part in the host's defence against infection; however, inflammation-induced coagulation could be detrimental if it is not adequately controlled and could lead to the clinical syndrome of disseminated intravascular coagulation. Plasma levels of anticoagulant vitamin-K-dependent protein C, vitamin-K-dependent protein S and antithrombin 3 are decreased in patients with acute severe melioidosis<sup>241,242</sup>. High ratios of thrombin–antithrombin complexes over plasmin- $\alpha$ 2-antiplasmin complexes (which reflect the consumption of clotting factors and activation (high ratios) or inhibition (low ratios) of fibrinolysis pathways) indicate a predominance of procoagulant mechanisms in melioidosis, and elevated levels of soluble endothelial protein C receptor (whose function is less clear compared with the antithrombotic and anti-inflammatory effects of membrane-bound endothelial protein C receptor) on hospital admission are associated with increased mortality<sup>243</sup>. Furthermore, mice deficient in plasminogen activator inhibitor 1 (which have increased fibrinolysis and, therefore, decreased fibrin deposition) show heightened susceptibility to *Burkholderia pseudomallei*<sup>244</sup>. Activated protein C and the protein C system seem to have a bidirectional role, with a minimal amount of activated protein C required to support an appropriate antibacterial host response, whereas overexpression leads to a harmful phenotype<sup>243</sup>. Interestingly, the cytoprotective effects of activated protein C are independent of its anticoagulant function. The  $\alpha$ 2-antiplasmin, a major inhibitor of fibrinolysis, protects from experimental melioidosis by limiting bacterial growth, inflammation, tissue injury and coagulation<sup>245</sup>. The urokinase-type plasminogen activator receptor, which also plays a crucial part in fibrinolysis, protects against melioidosis by facilitating the migration of neutrophils to the site of infection and subsequently enabling the phagocytosis of *B. pseudomallei*, further underlying the bidirectional role between coagulation and inflammation in melioidosis<sup>240</sup>.

**Box 5 |****Antibiotic therapy for treatment of melioidosis****Initial intensive therapy**

Initial intensive therapy should last for a minimum of 10–14 days and consists of ceftazidime (in wards) 2 g (50 mg kg<sup>-1</sup> up to 2 g in children (<15 years of age)) intravenous, 6-hourly; or meropenem (in intensive care units) 1 g (25 mg kg<sup>-1</sup> up to 1 g in children) intravenous, 8-hourly.

- Meropenem is the preferred initial intravenous therapy for neurological melioidosis (that is, in the presence of infection of the central nervous system), and the dose should be doubled.
- Consider adding trimethoprim–sulfamethoxazole (which has excellent tissue penetration) in the doses recommended for eradication therapy from the start of the initial intensive therapy in cases of neurological melioidosis, osteomyelitis and septic arthritis, skin and soft tissue infections and genitourinary infection including prostatic abscesses<sup>12,168</sup>.
- Long-term intravenous therapy ( 4–8 weeks) is recommended\* where possible for complicated pneumonia, deep-seated infection (including prostatic abscesses), neurological melioidosis, osteomyelitis and septic arthritis<sup>168,246</sup>.

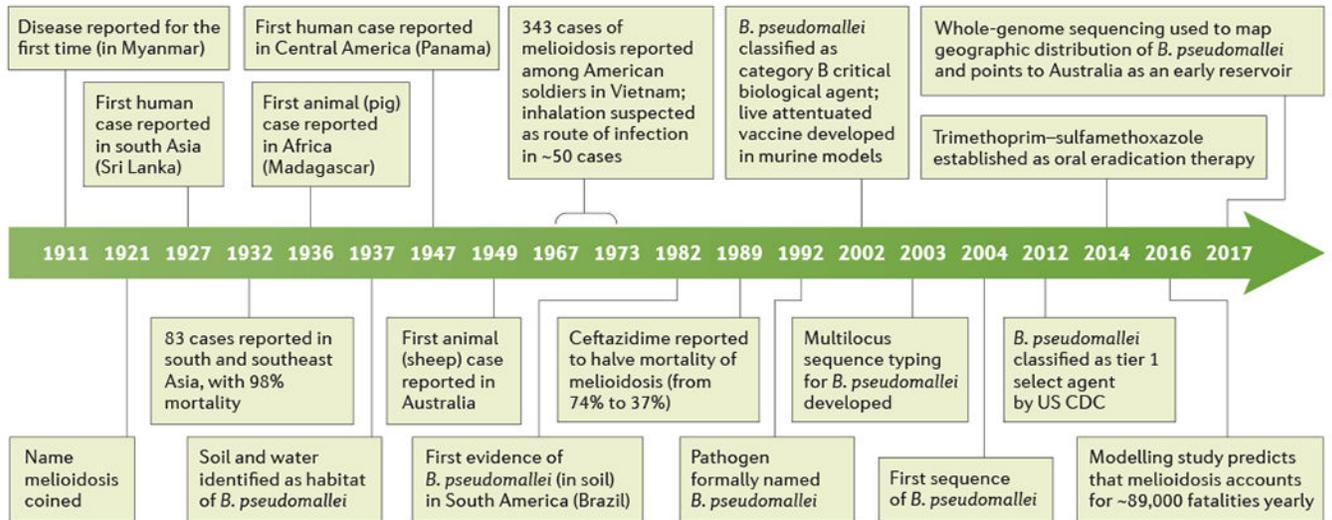
**Eradication therapy**

The eradication therapy should last for 3 months after the end of the initial intensive therapy and consists of trimethoprim–sulfamethoxazole<sup>‡</sup> 6 + 30 mg kg<sup>-1</sup> up to 240 + 1,200 mg in children, 240 + 1,200 mg in adults 40–60 kg and 320 + 1,600 mg in adults >60 kg orally, 12-hourly, and folic acid 5 mg (0.1 mg kg<sup>-1</sup> up to 5 mg in children) orally, daily.

- Longer eradication therapy ( 6 months) is recommended for neurological melioidosis and osteomyelitis.

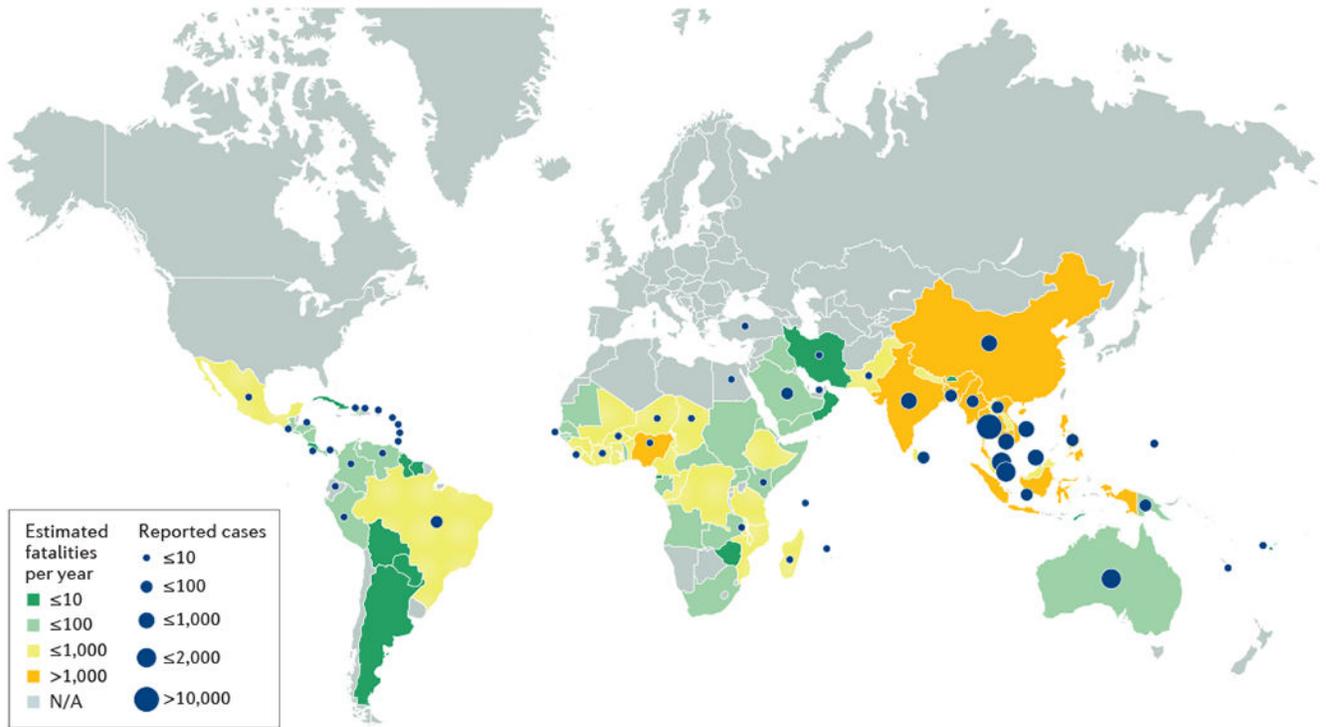
\*This treatment guidance is consistent with the most up-to-date recommendations by the International Melioidosis Society (<http://www.melioidosis.info>). Recommendations derived from Australian studies<sup>121,88</sup> and apply to resource-rich countries. In melioidosis-endemic regions, such prolonged intravenous therapy is often either not available or not affordable. Nevertheless, a minimum of 10 days of intravenous therapy is recommended for all individuals with melioidosis, except for those with localized skin disease without sepsis<sup>168,171</sup>.

‡The trimethoprim–sulfamethoxazole dose is usually expressed as separate doses of each individual drug.



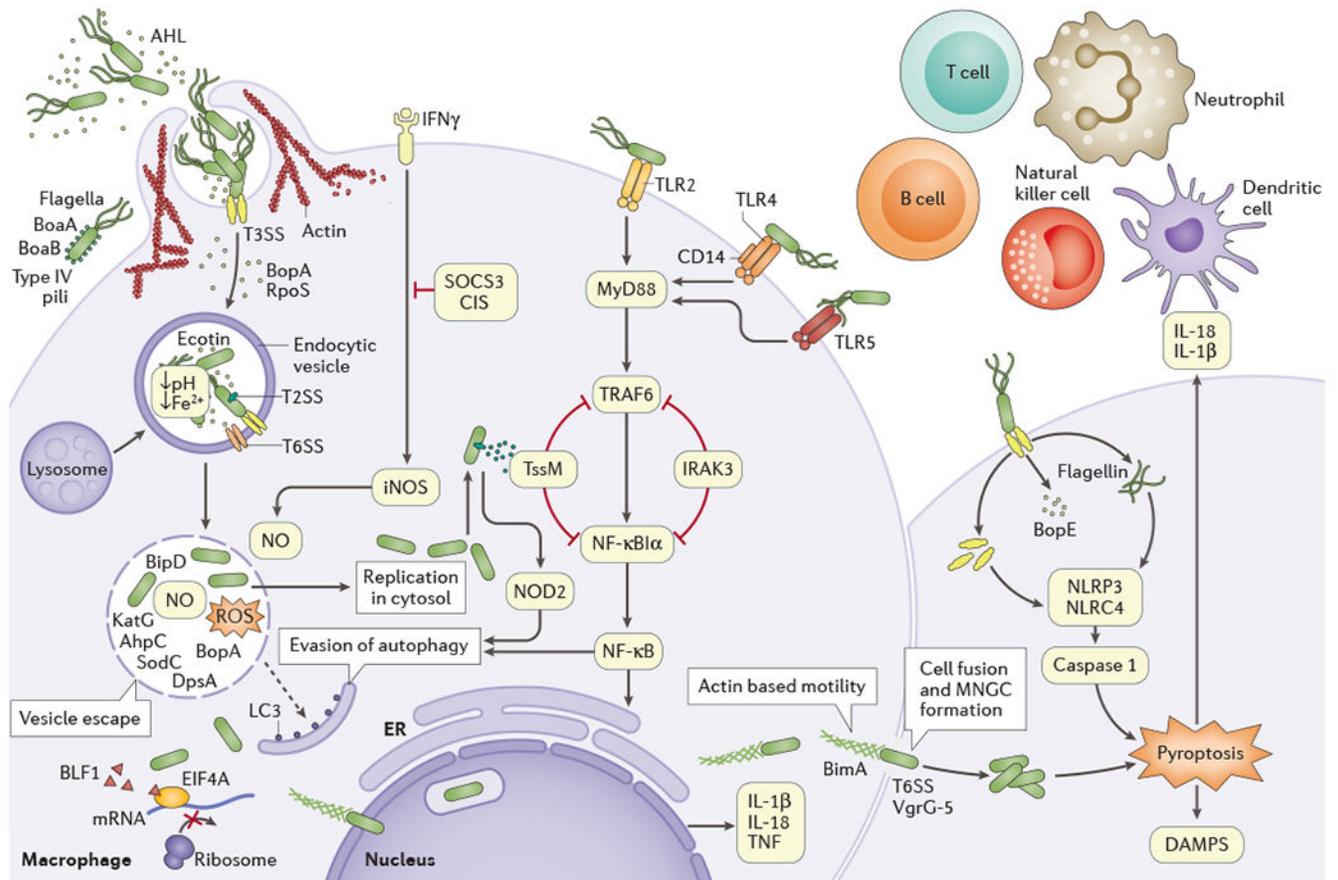
**Figure 1 | Milestones in the history of melioidosis.**

Melioidosis was first recognised in Rangoon in 1911 by the British doctor Alfred Whitmore and his assistant C. S. Krishnaswami, although the name of the disease was coined by Thomas Stanton and William Fletcher. From the time when the aetiological organism was first identified, it has been renamed many times: *Bacterium* (or *Bacillus*) *whitmori*, *Malleomyces pseudomallei*, *Loefflerella pseudomallei*, *Pfeifferella whitmori*, *Pseudomonas pseudomallei* and, finally, it was officially named *Burkholderia pseudomallei* in 1992. CDC, Centers for Disease Control and Prevention.



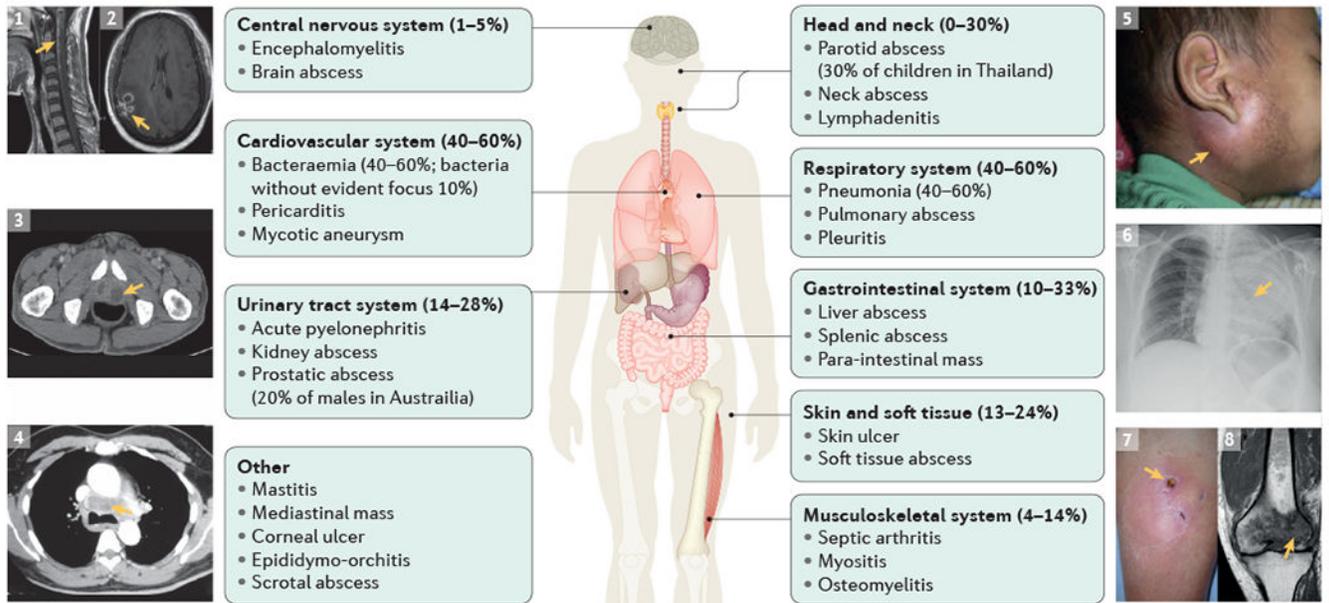
**Figure 2 |. Estimated mortality and reported cases of melioidosis.**

Only Australia, Brunei and Singapore have national surveillance data for melioidosis that are comparable to the estimates. Between 2010 and 2015, there were >100 culture-confirmed cases of melioidosis at a single hospital in Lao People's Democratic Republic yearly<sup>247</sup>, a number that supports the estimated 420 cases per year countrywide<sup>3</sup>. However, ~20,000 cases of melioidosis per year are estimated in Indonesia, but only 64 have been reported in the country since 1921 (REF. 248). A large difference between the numbers of predicted and observed cases is also observed in Bangladesh, Brazil, China, India and Nigeria<sup>3</sup>. This discrepancy could be due to limitations of the model, underuse of clinical microbiology laboratories<sup>206</sup>, lack of awareness of melioidosis and poor disease reporting systems. Based on data from REF. 3. N/A, not applicable.



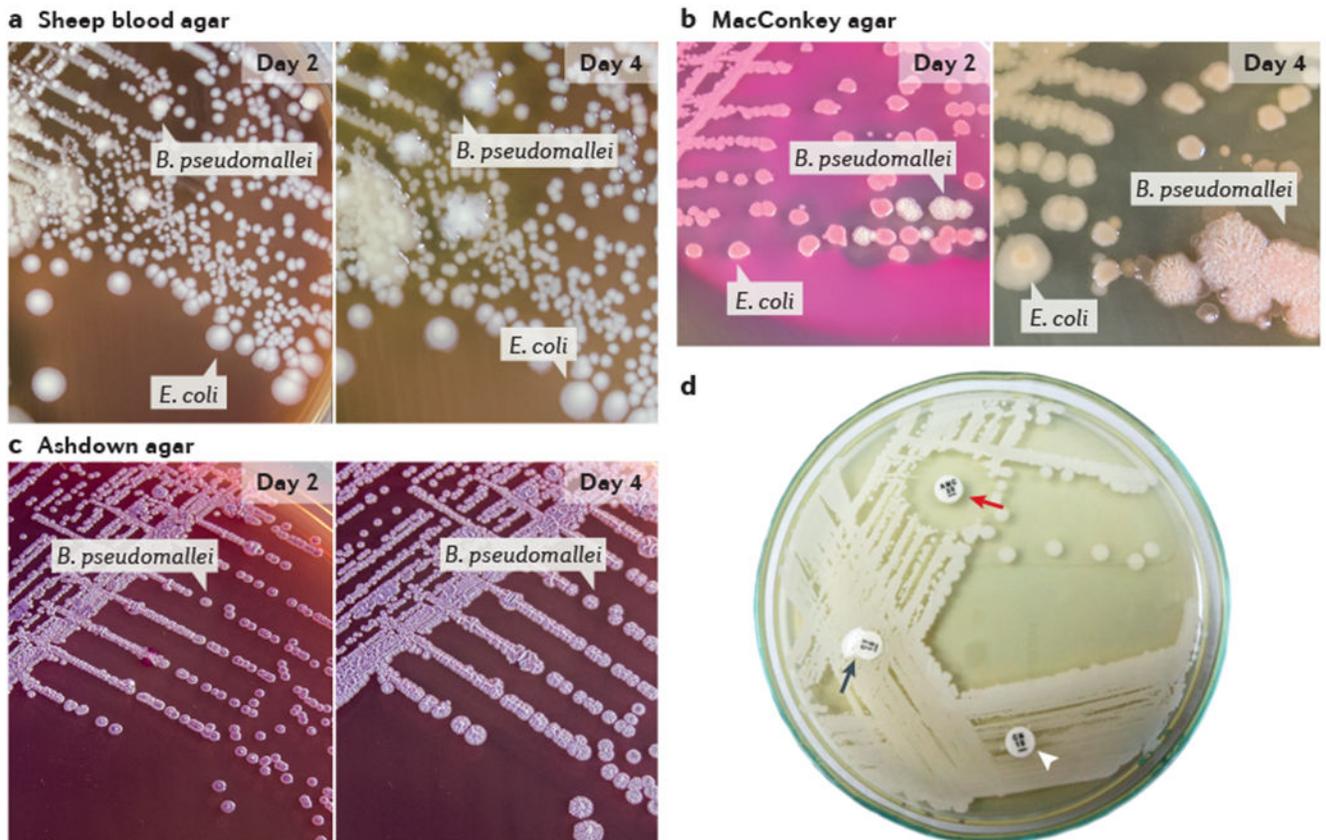
**Figure 3 | Schematic model of host-pathogen interactions and pathophysiology of melioidosis.** *Burkholderia pseudomallei* secretes *N*-acyl-homoserine lactones (AHL), which are signalling molecules involved in the quorum sensing machinery that is used to coordinate attacks against the host environment and biofilm formation. The type III secretion system (T3SS) effector proteins are necessary for invasion and escape from the endocytic vesicle; cell entry is aided by flagella, lipopolysaccharide (LPS), type IV pili and adhesins BoxA and BoaB. *B. pseudomallei* then quickly escapes the vesicle by lysing the membrane using T3SS, T6SS and T2SS. Metabolic flexibility (resistance to oxidative stress), resistance to antimicrobial cationic peptides and ecotin production enable bacteria to survive within an acidic endocytic environment. Effector protein BopA and translocator protein BipD further block sequestration in endocytic vesicles and prevent microtubule-associated proteins 1A/1B light chain 3B (LC3)-associated autophagy. Once free in the cytoplasm, *B. pseudomallei* replicates, induces the formation of actin-based membrane protrusions and can move via continuous polymerization of host cell actin at polar ends (a process regulated by autotransporter BimA), thereby facilitating spread to neighbouring cells, cell fusion and multinuclear giant cell (MNGC) formation. T6SS and the type IV secretion system (VgrG-5) are essential to this process. Toll-like receptors (TLRs) located on cell surfaces recognize pathogen-associated molecular patterns (such as LPS and flagella) and mediating nuclear factor- $\kappa$ B (NF- $\kappa$ B)-induced activation of the immune response, releasing pro-inflammatory cytokines IL-1 $\beta$  and IL-18. Intracellular inflammasome receptors such as NLR family

CARD domain-containing protein 4 (NLRC4) and NACHT, LRR and PYD domains-containing protein 3 (NLRP3) recognize bacterial virulence factors and damage-associated molecular patterns (DAMPs), triggering caspase-1-mediated pyroptosis and further release of IL-1 $\beta$  and IL-18. IL-18 further ensures protective interferon- $\gamma$  (IFN $\gamma$ ) production (mainly from natural killer cells). Neutrophils, dendritic cells, B cells and T cells are recruited towards the site of infection, and the complement and coagulation cascades are activated. AhpC, alkyl hydroperoxide reductase; BLF1, *Burkholderia* lethal factor 1; CIS, cytokine-inducible SH2-containing protein; DpsA, DNA starvation/stationary phase protection protein; EIF4A, eukaryotic initiation factor 4A; ER, endoplasmic reticulum; iNOS, inducible nitric oxide synthase; IRAK3, interleukin 1 receptor-associated kinase 3; KatG, catalase-peroxidase; MyD88, myeloid differentiation primary response protein; NF- $\kappa$ BI $\alpha$ , NF- $\kappa$ B inhibitor- $\alpha$ ; NO, nitric oxide; NOD2, nucleotide-binding oligomerization domain-containing protein 2; ROS, reactive oxygen species; RpoS, RNA polymerase  $\sigma$ -factor RpoS; SOCS3, suppressor of cytokine signalling 3; SodC, copper/zinc superoxide dismutase; TNF, tumour necrosis factor; TRAF6, TNF receptor-associated factor 6; TssM, type VI secretion system.



**Figure 4 |. Clinical manifestations of melioidosis.**

Examples of possible clinical presentations of melioidosis: an MRI of the brainstem and cervical spinal cord with inflammatory changes consistent with encephalomyelitis (arrow, part 1); a ring-enhancing lesion with surrounding oedema in the MRI image indicating cerebral abscesses (arrow, part 2); a CT image of prostatic abscesses (arrow, part 3); a CT image of a mediastinal mass (arrow, part 4); a child with tense parotitis (arrow, part 5); X-ray image of severe pneumonia (arrow, part 6); photo of a subcutaneous abscess (arrow, part 7); and an MRI image of osteomyelitis of the distal femur with surrounding inflammation (arrow, part 8). Clinical images 1–4, 6–8 courtesy of Bart J. Currie, Menzies School of Health, Australia. Clinical image 5 is reproduced with permission from (REF. 249), Elsevier.



**Figure 5 | Identification of *Burkholderia pseudomallei* colonies on three common types of agar.** Typical appearance of *Burkholderia pseudomallei* and *Escherichia coli* isolated from non-sterile clinical samples. Suspected clinical specimens and suspected bacterial colonies should be processed in a biological safety cabinet. **a** | *B. pseudomallei* forms creamy, non-haemolytic colonies that resemble a coliform after 2 days of incubation; by day 4, the colonies are covered by a slight metallic sheen and become dry and wrinkled. **b** | *B. pseudomallei* colonies resemble a colourless, non-lactose fermenting coliform after 2 days of incubation; by day 4, the colonies appear dry and wrinkled. **c** | After 2 days of incubation, the first visible *B. pseudomallei* colonies are pinpoint with a clear to pale pink colour; by day 4, they become darker pink to purple, flat, slightly dry and wrinkled with a definite metallic sheen. *E. coli* fails to grow because it is inhibited by gentamicin in the agar. **d** | Three-disc diffusion antibiotic sensitivity testing: *B. pseudomallei* is resistant to colistin (or polymyxin) (black arrow) and gentamicin (arrowhead) (although sensitive isolates exist in some areas) and sensitive to co-amoxiclav (red arrow). Parts **a–c** courtesy of Direk Limmathurotsakul, Premjit Amornchai and Vanaporn Wuthiekanun, Mahidol-Oxford Tropical Medicine Research Unit, Thailand. Part **d** courtesy of Vanaporn Wuthiekanun, Mahidol-Oxford Tropical Medicine Research Unit, Thailand.

Table 1

Selected virulence factors of *Burkholderia pseudomallei*

Gene	Antigen	Function	Refs
<b>Adherence</b>			
<i>pilA</i>	Type IV pilin subunit protein PiliA	<ul style="list-style-type: none"> <li>Temperature-dependent adherence and formation of microcolonies in some <i>Burkholderia pseudomallei</i> strains</li> <li>Intracellular motility</li> </ul>	42,50
<i>boxA</i>	Adhesins	<ul style="list-style-type: none"> <li>T3SS autotransporters</li> <li>Role in cell attachment and possibly intracellular replication</li> </ul>	48,54,250
<i>boxB</i>			
<i>bpaC</i>	Adhesin	<ul style="list-style-type: none"> <li>T3SS trimeric autotransporter ad hesin</li> <li>Protects from complement killing</li> <li>Involved in cell attachment, which could be cell-specific (for example, in ciliated mucosal epithelial cells)</li> </ul>	43,48
<i>flhC</i>	Flagellin structural component	<ul style="list-style-type: none"> <li>Required for flagellar assembly</li> <li>Polar tuft of 2–4 flagella allows temperature-independent motility</li> <li>Involved in cell adherence</li> </ul>	251,252
<b>Invasion</b>			
<i>bopE</i>	Guanine nucleotide exchange factor BopE	<ul style="list-style-type: none"> <li>T3SS effector</li> <li>Targets CDC42 and RAC1, inducing actin rearrangements and aiding cell invasion</li> </ul>	55
<i>bipB</i>	Translocator protein	<ul style="list-style-type: none"> <li>T3SS components</li> <li>Involved in endocytic vesicle survival, escape and cell invasion</li> </ul>	46,62
<i>bipC</i>	Effector protein		
<i>bipD</i>	Translocator protein		
<i>irfR</i>	Transcriptional activator protein IrfR	Mutants displayed reduced invasion	58
<b>Endocytic escape</b>			
<i>bopA</i>	Effector protein	<ul style="list-style-type: none"> <li>T3SS component</li> <li>Involved in endocytic vesicle membrane disruption and avoidance of autophagy</li> </ul>	77,78
<i>bsaQ</i>	T3SS structural component	Involved in endocytic vesicle escape, cell invasion and plaque formation	45,253
<i>bsaZ</i>	T3SS structural component	Implicated in endocytic vesicle escape and intracellular replication	46,254
<i>bsaU</i>	T3SS structural component	Involved in endocytic vesicle escape and early onset activation of the caspase 1 pathway in macrophages	115,255
<i>CHBP</i> ( <i>cif</i> homologue)	ATP and GTP binding protein	<ul style="list-style-type: none"> <li>T3SS structural component</li> <li>Delays host cell maturation, arresting cycle in G2-M and impeding apoptosis</li> </ul>	13,43,62,256
<b>Intracellular survival</b>			
<i>purM</i>	Phosphoribosylformylglycinamide cyclo-ligase	<ul style="list-style-type: none"> <li>Purine biosynthetic pathway</li> <li>Knockout models show decreased intracellular replication</li> </ul>	42,255
<i>purN</i>	Phosphoribosylglycinamide formyltransferase		

Gene	Antigen	Function	Refs
<i>sodC</i>	Superoxide dismutase	Resistance to oxidative stress	70–73
<i>katG</i>	Catalase-peroxidase		
<i>ahpC</i>	Alkyl hydroperoxide reductase		
<i>dpsA</i>	DNA starvation and stationary phase protection protein		
<i>rpoE</i>	RNA polymerase $\sigma$ -factor RpoE	<ul style="list-style-type: none"> <li>Biofilm formation, heat stress response via RNA polymerase <math>\sigma</math>-factor RpoH-regulated heat shock proteins, oxidative and osmotic stress</li> <li>Mutants show reduced intracellular survival in macrophages</li> </ul>	44,257
<i>virAG</i> locus	Two-component regulatory system	<ul style="list-style-type: none"> <li>Regulates T6SS transcription</li> <li>Sensor of the iron-limiting environment of endocytic vesicles and histidine kinase</li> <li>Upregulated at acidic pH, affecting T6SS secretion process</li> </ul>	13,229
<i>rpoS</i>	RNA polymerase $\sigma$ -factor RpoS	<ul style="list-style-type: none"> <li>Suppresses iNOS activity by upregulating SOCS3 and CIS cytokines</li> <li>Could play a part in regulating genes involved in macrophage fusion</li> </ul>	68,258
<b>Actin-based motility</b>			
<i>bimA</i>	T5SS autotransporter	<ul style="list-style-type: none"> <li>Escape from phagosome and actin tail formation</li> <li>Encephalomyelitis strongly correlated with <i>BimA<sub>Bm</sub></i> allele</li> </ul>	80,81,83
<b>Multinuclear giant cell formation</b>			
<i>hcp1</i>	Hcp1 family T6SS effector	<ul style="list-style-type: none"> <li>Role in cell fusion and macrophage cytotoxicity</li> <li>Induces production of IL-10 and TGF<math>\beta</math></li> </ul>	259–262
<i>vgrG5</i>	Rhs element Vgr protein	<ul style="list-style-type: none"> <li>T6SS effector (tail-spike tip)</li> <li>Functionally conserved across <i>Burkholderia</i> spp.</li> </ul>	263
<b>Others</b>			
<i>tssM</i>	TssM	<ul style="list-style-type: none"> <li>T2SS effector (a deubiquitinase)</li> <li>Targets TRAF3, TRAF6 and NF-<math>\kappa</math>B inhibitor-<math>\alpha</math> inhibit type 1 interferon and NF-<math>\kappa</math>B pathways</li> </ul>	47,264
<i>wcb</i> operon	Capsular polysaccharides	<ul style="list-style-type: none"> <li>Protection from C3b complement and normal human serum</li> <li>Protection from environmental dangers</li> <li>Biofilm production, not essential for survival but contributes to chronic infection and latency</li> </ul>	265–267
Various genes (including <i>waaF</i> , <i>lytB</i> (also known as <i>ispH</i> ) and others)	Lipopolysaccharides	<ul style="list-style-type: none"> <li>Resistance to normal human serum and cationic peptides</li> <li>Reduced minimal pyrogenic lethal toxicity (measure of inflammatory response) and macrophage activation</li> <li>Length, number and position of fatty acyl chains can affect LPS bioactivity and vary between virulent strains</li> </ul>	44,111,112,268
<i>luxI</i> and <i>luxR</i> homologues	N-Acyl homoserine lactones	<ul style="list-style-type: none"> <li>Quorum sensing (also mediated by a second system using HMAQ)</li> <li>Upregulate transcription of genes simultaneously within a bacterial population involved in colonization, longer survival and higher LD<sub>50</sub></li> <li>Regulation of siderophore synthesis</li> </ul>	269,270
BLF1	<i>Burkholderia</i> lethal factor 1	<ul style="list-style-type: none"> <li>Glutamine deamidase, similar to <i>Escherichia coli</i> cytotoxic necrotizing factor</li> <li>Inversibly interferes with initiation of translation by inactivating EIF4A and thereby inhibiting recruitment of 40S ribosomal subunit and protein synthesis</li> </ul>	271,272

Gene	Antigen	Function	Refs
Multiple genes upregulated in tandem	Morphotype switching	<ul style="list-style-type: none"> <li>Cell cytoskeleton alteration and cell death. Concentrations as low as <math>2.5 \times 10^{-7}</math> M can cause cell death with molecular turnover (a measure of enzymatic efficiency) like that of ricin</li> <li>Seven morphotypes, wrinkled type 1 predominates</li> <li>Strain differences in colony morphology phenotypically lead to changes in biofilm production, secreted enzymes and motility, hence influencing intracellular survival, lethality and persistence</li> </ul>	273

This is not a comprehensive list: the *B. pseudomallei* virulence factors shown are well-studied representative examples involved throughout the life cycle of *B. pseudomallei*. CDC42, cell division control protein 42 homologue; CIS, cytokine-inducible SH2-containing protein; EIF4A, eukaryotic initiation factor 4A; HMAQ, 4-hydroxy-3-methyl-2-alkylquinolone signalling molecules; iNOS, inducible nitric oxide synthase; NF- $\kappa$ B, nuclear factor- $\kappa$ B; LD50, median lethal dose (dose lethal to 50% of animals tested); LPS, lipopolysaccharide; RAC1, Ras-related C3 botulinum toxin substrate 1; SOCS3, suppressor of cytokine signalling 3; TSSS, type V secretion system; TGFB $\beta$ , transforming growth factor- $\beta$ ; TNF, tumour necrosis factor; TRAF3, TNF receptor-associated factor 3; TssM, type VI secretion system ATPase and inner membrane protein.