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Burkholderia pseudomallei and melioidosis

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Abstract

Burkholderia pseudomallei, the causative agent of melioidosis, is found in soil and water of tropical and subtropical regions globally. Modelled estimates of the global burden predict that melioidosis remains vastly under-reported, and a call has been made for it to be recognised as a neglected tropical disease by the World Health Organization. Severe weather events and environmental disturbance are associated with increased case numbers, and it is anticipated that in some regions cases will increase in association with climate change. Genomic epidemiological investigations have confirmed *B. pseudomallei* endemicity in newly recognised regions including the southern United States. Melioidosis follows environmental exposure to *B. pseudomallei* and is associated with comorbidities that affect the immune response such as diabetes, and with socioeconomic disadvantage. Several vaccine candidates are ready for phase 1 clinical trials. In this Review, we explore the global burden, epidemiology, and pathophysiology of *B. pseudomallei* as well as current diagnostics, treatment recommendations and preventative measures, highlighting research need and priorities.

[H1] Introduction

Burkholderia pseudomallei is an environmental Gram-negative bacterium, and the causative agent of melioidosis in humans and animals[1]. Infection occurs following environmental exposure via percutaneous inoculation, inhalation, or ingestion[2,3]. The majority of cases are sporadic, however small human case clusters have been reported in association with contaminated products, water supplies, or environments[4-6]. Person-to-person and zoonotic transmission are extremely rare. Animal outbreaks have been reported in zoos and agricultural facilities, with exotic animals imported from non-endemic to endemic areas particularly at risk[7,8].

Most individuals exposed to *B. pseudomallei* do not develop melioidosis, however diabetes and other conditions that impair innate and adaptive immune responses are important risk factors[9,10]. An array of virulence factors allows the bacterium to adhere to, invade, and multiply within host cells, with intracellular survival as a key mechanism of immune evasion[1]. Mortality ranges from under 10% to 40% or higher[9-11], and is mostly determined by presence of clinical risk factors and access to laboratory diagnosis, appropriate antimicrobial therapy, and intensive care support. Due to its pathogenic potential and limited antimicrobial treatment options, *B. pseudomallei* is classified as a Tier 1 Select Agent by the Centers for Disease Control and Prevention in the United States.

Most reported melioidosis cases occur in Southeast Asia and northern Australia, however the known area of endemicity is expanding, with cases increasingly being reported in the Pacific, South Asia, Africa, and the Americas[12,13]. It is predicted that melioidosis incidence will increase in some regions with climate change[7]. Unmasking of endemicity in the southern United States[14] and in Southeast Queensland, Australia[15], has recently been reported, the latter in consecutive very wet years associated with the La Niña phase of the El Niño-Southern Oscillation.

Modelling of the global burden suggests that melioidosis is massively underdiagnosed[12], attributed to limited access to laboratory diagnostics and lack of clinical awareness in some endemic areas[16]. People living in rural and remote regions are disproportionately affected, including agricultural workers in Southeast Asia[17], and First Nations peoples in northern Australia[18]. Low socioeconomic status is associated with comorbidities that increase melioidosis risk and is an independent risk factor for death due to melioidosis[19]. For these reasons, a call has been made for recognition of melioidosis as a neglected tropical disease

by the World Health Organization (WHO), with the aim of improving melioidosis surveillance, awareness, diagnosis, and management[16].

Melioidosis cannot be differentiated from other causes of community-acquired infection based on clinical or radiological features alone. There is enormous diversity of melioidosis clinical presentations, influenced by bacterial inoculating dose, mode of acquisition, host risk factors, and probably differential virulence of infecting *B. pseudomallei* strains. Predisposing comorbidities in the host are the main determinant of both disease severity and mortality. Around half of patients have pneumonia on presentation, over 50% have bacteremia, and over 20% develop septic shock requiring intensive care management often with inotropic and ventilatory support[9,10]. In contrast, skin infections usually present as a single lesion without sepsis and are the commonest presentation seen in children in Australia[20], while parotid abscesses are the most common presentation in children in some Southeast Asian countries[21]. Genitourinary sepsis with prostatic abscesses is especially common in males in Australia, and other presentations include abscesses in the liver or spleen, bone and joint infections, encephalomyelitis, meningitis or brain abscess, and bacteremia without an evident focus[9,10].

In this Review, we discuss the epidemiology of melioidosis including its global burden and distribution, *B. pseudomallei* virulence and the host immune response, and current recommendations for diagnosing, treating, and preventing melioidosis. We outline future research priorities focused on understanding and mitigating the effects of climate change and environmental disturbance on melioidosis incidence and decreasing mortality through improved diagnosis, treatment, and prevention.

[H1] Epidemiology

[H2] Clinical epidemiology

Infections with *B. pseudomallei* in humans or animals result from the exposure of the host to bacteria in soil or water. Infection occurs via percutaneous exposure, inhalation, aspiration, or ingestion[22]. Epidemiological data support both a shift to more inhalation during severe weather events with wind and rain[2] and a higher proportion of cases from ingestion in regions with unchlorinated water supplies[23]. The high seropositivity seen in northeast Thailand, with seropositivity rates reaching 50% in young children[24], may also reflect ingestion of *B. pseudomallei* however the proportion of antibody-generating

exposures arising from different infecting routes is not known. The Thai seropositivity rates contrast dramatically with the low seropositivity seen in northern Australia despite similar melioidosis incidence[25]. Whether frequent exposure to *B. pseudomallei* in endemic regions confers any immunity with protection from severe clinical disease remains unknown. Serology studies support that most infections with *B. pseudomallei* are asymptomatic, and the proportion that develops latent infection is not known. The likelihood of developing melioidosis from the activation of *B. pseudomallei* from latency is well recognized and was referred to as 'The Vietnam time-bomb'. However, such activation from latency has been very uncommon (3% of cases in one series[9]), and many of the older published cases described as activation from latency were not actually asymptomatic prior to melioidosis diagnosis, but instead had histories of more chronic and/or relapsing illness, such as for 24 years in one Australian case[26] and for 26 years in one United States case[27]. Such historical cases likely reflect unrecognised chronic and/or recrudescent melioidosis, which would today be diagnosed earlier with appropriate sampling and current laboratory protocols. The longest documented asymptomatic latency period before activation is 29 years in a U.S. Vietnam veteran[28].

In the Darwin prospective melioidosis study, only 29 (3%) of 1,148 consecutive culture-confirmed melioidosis patients were considered possible activation from latency[9]. Most cases (88%) were acute illness from recent infection. Where a likely infecting event was recalled by the patient, the incubation period was 1-21 days (median 4 days)[9]. Chronic melioidosis, defined as symptoms being present for 2 months or longer, occurred in 9% of cases, with predominantly subacute pulmonary disease often mimicking tuberculosis, or non-healing skin infection.

Diabetes was the commonest clinical risk factor for melioidosis in Australian and Thai studies[9,10,29]. Hazardous alcohol consumption, chronic kidney disease, chronic lung disease, immunosuppressive therapy (most commonly corticosteroids) and thalassemia with iron overload are also well recognized risk factors. Patients with cystic fibrosis are at high risk of melioidosis and are advised to avoid travel to or exposure in melioidosis-endemic regions[30]. No evident clinical risk factors were found in 16% (Australia)[9] and 36% (Thailand)[29] of cases.

[H2] Global burden and distribution

Melioidosis is highly endemic in Southeast Asia and northern Australia, where *B. pseudomallei* is commonly found in the environment. Nonetheless, melioidosis is now considered endemic in many tropical countries worldwide.

A modelling study estimated that there were approximately 165,000 melioidosis cases causing 89,000 deaths per year globally in 2015[12]. A subsequent study published in 2019 estimated the global burden of melioidosis as 4.64 million disability-adjusted life-years (DALYs), which is higher than that of many neglected tropical diseases officially listed by the WHO[31]. The estimated number of DALYs per 100,000 people varied considerably between countries due to differences in both incidence and case fatality rates – for example, from 8.7 in Australia to 212.6 in Thailand[31].

Isolation of *B. pseudomallei* from both clinical and environmental samples provides strong evidence of melioidosis endemicity. However, exploring the presence of *B. pseudomallei* in a country where microbiology facilities are limited is challenging. Improving the identification of *B. pseudomallei* by using a simple laboratory algorithm with disc diffusion susceptibility testing to amoxycillin-clavulanate (susceptible), gentamicin (resistant), and colistin (resistant) has proven useful in Vietnam[32]. A combination of environmental sampling and a prospective serological and microbiological surveillance could also be useful. Unfortunately, isolation of *B. pseudomallei* from environmental samples can be difficult[33], but molecular diagnostics can be used to screen and support the culture for *B. pseudomallei*[34,35]. Based on the evidence from 2015[12], melioidosis was considered endemic in at least 48 countries spanning southeast and south Asia, Australia, the Pacific and Indian Ocean Island nations, sub-Saharan Africa, central and south America, and the Caribbean.

The global map presented in FIG. 1 documents human cases, animal cases, and presence of *B. pseudomallei* in the environment from January 1910 to September 2022, and includes evidence of melioidosis in 12 new countries: Benin, Cameroon, Democratic Republic of Congo, Eritrea, Federal States of Micronesia, Ghana, Mali, Nepal, Nicaragua, Saint Kitts and Nevis, Trinidad and Tobago, and most recently the southern United States[13]. The updated method for determining the strength of evidence for melioidosis endemicity, and references for newly reported endemic areas are included in the Supplementary information.

Modelling predicted that the southern United States was receptive to endemicity for *B. pseudomallei*[12]. Genotyping of *B. pseudomallei* from a 2018 patient from Texas suggested local acquisition[36] and in 2022 *B. pseudomallei* was recovered from soil and water in

Mississippi, and the isolates were linked by genotyping to two locally-acquired melioidosis cases, which confirmed for the first time that melioidosis is endemic in the United States[14].

[H2] Environmental niches, seasonality, and climate

B. pseudomallei grows best in wet, acidic, low salinity, nutrient-deplete soil with low carbon levels[12,37-39], but is able to persist for many years during drought conditions, including desert and temperate environments[40,41]. It has been found in association with both clayish and sandy soils, with evidence showing that it grows best in deep clay silt layers with high porosity and hydraulic conductivity[37,42,43]. The effect of iron content on *B. pseudomallei* growth depends on bioavailability, oxidation state and other physicochemical properties[38,44,45]. Consensus guidelines for environmental sampling recommend collecting at least 100 soil specimens located 2.5 to 5 meters apart from each other, from an area of approximately 2,500 square meters, at a depth of 30 centimeters[46]. However, the layers in which *B. pseudomallei* is found will vary in different locations depending on physicochemical properties, and in some areas it may be present in much deeper, nutrient-deplete layers with year-round water[43].

B. pseudomallei is found in the rhizosphere around the roots of grasses including rice[42], in the aerial portion of grasses[47], and in faecal matter of grazing livestock and native animals, which may be vectors for importation of *B. pseudomallei* into non-endemic areas[48]. Isolation of *B. pseudomallei* in the environment is associated with land disturbance including agricultural activities such as rice farming[42,47,49,50], and increased case numbers have been observed during periods of major suburban construction work[9,51,52].

In water, *B. pseudomallei* can be isolated from turbid drains and rivers which act as conduits for dispersal of run-off from their catchments[51,53,54], in ground-water seeps connected to underground aquifers[55], and from a large proportion of rural bores (wells)[56]. Chlorination and ultraviolet light are effective means of killing *B. pseudomallei* in water[5,6,45,57].

B. pseudomallei has been isolated from air samples during severe weather events, and aerosolization may contribute to dispersal in the environment. However, long-range travel by this route is considered unlikely due to the intolerance of the bacteria to ultraviolet light[58,59].

The relationship between rainfall and melioidosis cases is well established, with most cases in endemic regions occurring during periods of heavy rainfall in the monsoonal wet

season[9,17]. During such periods, it is hypothesised that the bacterium is brought to the surface as the water table rises, where it then proliferates[43,60]. In northern Australia, increases in rainfall, groundwater, dew point, cloud cover, and maximum temperature are associated with increased melioidosis cases – with peak incidence during the La Niña phase of the El Niño-Southern Oscillation[61]. In Laos and Cambodia, increased humidity and wind speed have been found to be associated with increased melioidosis cases[62]. In some regions it is predicted that there will be an increase in the frequency and severity of extreme weather events as the climate changes; depending on human activities and environmental disturbance associated with agriculture and construction, it is very likely there will be an associated increase in melioidosis cases[7,61,63].

[H2] Genomic epidemiology

B. pseudomallei has an approximately 7.24 Mega base pairs (Mbp) genome with a guanine-cytosine (GC) content of ~68%, comprising two circular chromosomes 4.07 and 3.17 Mbp in size[64]. Due to extensive recombination and horizontal gene transfer, there is substantial variability in gene content between strains[65]. This diversity occurs in genomic islands mostly inserted into transfer RNA (tRNA) genes, which vary in site, number, and gene content, and contribute to *B. pseudomallei*'s open pangenome[66-68]. The extent to which this genomic variation contributes to differences in bacterial survival and virulence is not known. Allelic variation in a putative secreted adhesin gene (*BPSL1661*) is hypothesised to promote survival in nutrient-deplete conditions[69,70], while the *Burkholderia mallei*-like allele of the autotransporter protein BimA (found in some Australian and South Asian isolates) is strongly associated with encephalomyelitis presentation[71,72]. A genome-wide association study comparing clinical and environmental isolates identified toxin and adhesin gene loci associated with infection, however this study may not have adequately accounted for sampling bias and there was little overlap in the results from the two analysis methods employed[73].

Due to its environmental niche, *B. pseudomallei* populations are geographically restricted and long-range transmission is rare[74]. Australian strains are most diverse, with phylogeographic analyses supporting an Australian origin with subsequent dispersal to Asia, Africa, and the Americas[75,76]. This phylogeographic restriction can assist with investigating the source of melioidosis cases. For example, *B. pseudomallei* genomes from two

melioidosis cases in Texas, United States, clustered together and with other isolates from the Americas, supporting local acquisition[36]. Such phylogeographic comparisons[77] played a central role in refuting a previous hypothesis of acquisition for one Texas case, which was incorrectly attributed to infection 62 years earlier during World War II[78]. Human and animal melioidosis case clusters occasionally occur in association with contaminated products, water supplies, or environments, with environmental and clinical *B. pseudomallei* genomic sequences demonstrating distances of 0–1 single nucleotide polymorphism where a source is implicated[4-6,79,80]. A core genome multilocus sequence typing scheme has been developed as an alternative method for genome sequence comparisons[81]. In some instances, such genomic epidemiological investigations have enabled targeted public health intervention to address the source, such as installation of an ultraviolet light water filter, or recall of a contaminated product[4,6]. However, this does not apply to the vast majority of cases, which are sporadic.

[H1] Pathophysiology

[H2] Virulence factors and intracellular lifestyle

B. pseudomallei employs multiple mechanisms to escape antimicrobial defenses and is capable of both overwhelming the host leading to acute severe infection, and of hiding within the host for years evading the host immune response. To achieve this, *B. pseudomallei* has a formidable armoury of virulence factors (Supplementary Table 1). These help the bacterium to consecutively adhere to and invade host cells, multiply within them, infect neighbouring cells, and overcome antimicrobial host defence systems.

Key virulence factors include several secretion systems (*B. pseudomallei* has at least three type III secretion systems (T3SSs)), which deliver bacterial effector molecules into the host cytoplasm. The six known type VI secretion systems (T6SSs) are implicated in intracellular survival and competition with bacterial communities (FIG. 2). Ingeniously, *B. pseudomallei* uses host glutathione to modulate its virulence; for instance, the virulence-associated T6SS can be activated by membrane-bound histidine kinase sensor VirA by increased glutathione levels[82]. *B. pseudomallei* harbours at least 11 autotransporter proteins including Burkholderia intracellular motility A (BimA) which has been implicated in actin polymerization and motility promoting cell-to-cell spread[83]. BimA has been associated with central nervous system infection in murine models of melioidosis and the $bimA_{Bm}$ allele of *B.*

pseudomallei is related to neurological manifestations and worse outcomes[72]. Other notable virulence factors include the capsular polysaccharide, the lipopolysaccharide (LPS), flagella, and Burkholderia lethal factor 1[1,84-86] (Supplementary Table 1). In addition, recent studies investigating the metabolome of *B. pseudomallei* have identified novel virulence factors such as malleicyprols which show high toxicity in in vitro cell assays due to their ability to form a cyclopropanol warhead that can form β-keto radicals[87]. It should be noted, however, that the relative importance of any of these individual virulence factors for human disease remains ill-defined. Attenuated virulence associated with large-scale genome reduction has occurred in rare cases of long-term *B. pseudomallei* pulmonary infection and carriage, with immune evasion and treatment failure in the setting of severe bronchiectasis[88,89].

The ability of *B. pseudomallei* to survive intracellularly[90] explains the ability for latency and is a key aspect of the pathogenesis of melioidosis. *B. pseudomallei* can replicate in both phagocytic and non-phagocytic cells. After cell invasion, the bacterium can escape endocytic vacuoles and infect other cells through actin-based membrane protrusions. This can lead to direct cell-to-cell spread and contributes to the formation of multinucleated giant cells. Recent work using single-cell transcriptomics has described the dynamic alterations in bacterial gene expression during the transit of *B. pseudomallei* in host cells thereby identifying hypothetical proteins important for attachment, cytoskeletal modulation and evasion of autophagy[91]. These proteins could be exploited as novel therapeutic targets or vaccine candidates.

[H2] Host response against B. pseudomallei

Human defense against clinical melioidosis following exposure to *B. pseudomallei* depends on multiple layers of the immune system (BOX 1). The pathogen-associated molecular patterns (PAMPs) of *B. pseudomallei*, such as LPS, peptidoglycan, flagellin and its DNA, are sensed through a range of pattern recognition receptors (PRRs)[86,92]. Key Toll-like receptors (TLRs) involved in the host response against *B. pseudomallei* include TLR1, TLR2, TLR4 and TLR5 (reviewed in ref.[93]), and genetic variants in TLR4 and TLR5 in humans have been associated with susceptibility to melioidosis[94,95]. In addition, studies have shown that the Nod-like receptors (NLR) NLRC3 and NLRC4, which regulate pyroptosis (the highly inflammatory form of programmed cell death upon infection with intracellular pathogens) and the production of interleukin (IL)-1β and IL-18, are important for inflammasome-

mediated resistance to melioidosis in mice[96-98], and that there is an association between a nucleotide binding oligomerization domain 2 (NOD2) polymorphism and human melioidosis[99]. The *B. pseudomallei* T3SS inner rod protein BsaK was identified as an early activator of NLRC4-dependent caspase-1 processing, pyroptosis and IL-1b secretion thereby contributing to *B. pseudomallei* virulence in a murine model on infection[100].

PRRs activation leads to the recruitment of activated neutrophils towards the site of infection, playing a key role in early bacterial containment[101], in addition to macrophages and lymphocytes. Besides leukocytes, parenchymal cells, such as epithelial and endothelial cells are also important in the early response to melioidosis[102-104]. As with any severe infection, patients with melioidosis show signs of both hyperinflammation and immune suppression, two seemingly opposite reactions that involve partially different cell types and organ systems[105,106]. Likely, this disturbed immune response is not only the result of persistent stimulation by the virulence factors of *B. pseudomallei*, but also by the release of "damage-associated molecular patterns" (DAMPs), which are molecules derived from host cells that are released into the extracellular environment upon injury. DAMPs can trigger many of the PRRs that also sense PAMPs, giving rise to a vicious cycle with sustained immune activation and dysfunction. A prime example is calprotectin (S100A8/S100A9), a bioactive pro-inflammatory antimicrobial heterodimer that can activate TLR4 and, although not unique to melioidosis, has been shown to be an indicator of melioidosis disease activity in patients[107].

B. pseudomallei is a potent inducer of neutrophil extracellular traps (NETs), which are composed of histones, DNA and proteases and are released by neutrophils in order to ensnare and kill *B. pseudomallei*[108]. Patients with melioidosis display highly increased levels of NET-related components which further amplify the inflammatory response. The *B. pseudomallei* virulence factors T3SS and capsular polysaccharide I (CPS-I) have been shown to play a role in evading NETosis, which might explain why NETosis (NET activation and release) *per se* does not protect against bacterial dissemination in a mouse model of melioidosis[109].

The complement system, which is activated upon exposure to PAMPs and DAMPs and initiated by complement components C1q, mannose-binding lectin and ficolins, is strongly activated during melioidosis as was recently demonstrated in a non-human primate model[110]. *B. pseudomallei* however is resistant to human serum, indicating that the

bacterium is able to prevent the formation of significant levels of complement, most notably its terminal cascade product membrane attack complex (MAC), on their surface[110]. Earlier work had shown that the O-antigenic polysaccharide (O-PS) moiety of *B. pseudomallei* LPS is required for this serum-resistance phenotype but it is likely that other yet to be described evasion strategies also play a role[111].

Melioidosis is associated with strong activation of coagulation, which together with an impairment of endogenous anticoagulant mechanisms results in a net procoagulant state and an increased risk for microvascular thrombosis[103,112]. Recent insights show that platelets also play an important protective role in innate immune response against *B. pseudomallei* and the maintenance of vascular integrity[113]. Low platelet counts are independently associated with mortality in patients with melioidosis[113] and thrombocytopenic mice demonstrate an impaired host defence against *B. pseudomallei*[113]. Platelets do not seem to directly influence the bacterial growth of *B. pseudomallei*, but, during severe infections with a septic response, platelets also likely contribute to the development of organ failure by enhancing leukocyte recruitment and hyperinflammation, contributing to vaso-occlusive thrombi development in the microvasculature, and by direct cell toxic effects of platelets and platelet derived microparticles[113].

The importance of protective adaptive immunity has been demonstrated by the finding that survivors of melioidosis have elevated antibody, CD4 and CD8 T-cell mediated interferon (IFN)-γ responses to *B. pseudomallei*[114-116] and individual proteins[117-119] when compared to non-survivors. This is underscored by experimental rodent studies showing that humoral and cell-mediated immunity is essential for protection against *B. pseudomallei*[120,121]. In humans, specific immunoglobulin G (IgG) antibodies play a significant role in protection from lethal melioidosis[122,123]. Of interest, recent work suggested that environmental exposure to low-virulence *Burkholderia* strains such as *B. thailandensis* might build cellular immunity to *B. pseudomallei*[124].

[H1] Diagnosis, Treatment and Prevention

[H2] Culture, identification, sensitivity testing, direct detection, and serology

Isolation of *B. pseudomallei* from any site is diagnostic of melioidosis, and culture remains the gold standard diagnostic method. Collection of appropriate clinical specimens is crucial

for an accurate diagnosis; these include blood cultures, sputum, and urine, and depending on the presentation swabs, pus, and fluids from normally sterile sites. Body fluids including urine should be centrifuged, and the pellet cultured. Throat and rectal swabs can also be cultured in selective liquid media and may be helpful when there is difficulty obtaining a sputum specimen. Consensus guidelines recommend handling *B. pseudomallei* within a class II biological safety cabinet, ideally in a biosafety level 3 facility[125]; however, the risk of laboratory-acquired infection is extremely low and the organism is handled on the bench in many endemic settings[126].

B. pseudomallei grows well on standard media including 5% horse or sheep blood and chocolate agar at 35-37°C in air, but colonies may not be apparent until 48 hours and can be overgrown by commensal organisms in specimens from nonsterile sites[127]. *B. pseudomallei* will grow on MacConkey agar, although other selective media are preferable. Ashdown's agar contains gentamicin and crystal violet as selective agents and is widely used in endemic areas. *Burkholderia cepacia* selective agar is an excellent alternative and includes crystal violet, polymyxin B, gentamicin, and vancomycin antibiotics. Ashdown's broth contains crystal violet and colistin and can be used for inoculation of swabs from nonsterile sites as well as for environmental samples.

B. pseudomallei colonies are small, creamy, and have a metallic sheen, subsequently becoming dry and wrinkled; on Ashdown's medium they have a purple colour. *B. pseudomallei* is motile, can have a Gram-negative rod-shaped safety pin appearance on staining, and is oxidase positive and indole negative. Latex agglutination with monoclonal antibody against *B. pseudomallei* exopolysaccharide is a useful bench test to aid identification[128,129]. The antimicrobial susceptibility pattern can be used as an adjunct to identification; *B. pseudomallei* is intrinsically resistant to gentamicin and colistin, but susceptible to amoxicillin-clavulanate[32,130]. Gentamicin-susceptible isolates are rare but found sporadically, and predominate in Sarawak, Malaysia[131].

Misidentifications can occur using the VITEK® 2 GN ID card (bioMérieux) and API® 20 NE (bioMérieux) test, with the most common incorrect identification being *Burkholderia cepacia* complex[130,132,133]. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) provides much faster identification than the biochemical methods described above, but is limited by current commercial databases[134,135]. Current standard diagnostic databases for use with the two commercially available MALDI-TOF MS

systems, the Bruker Microflex Biotyper and the bioMérieux Vitek MS, do not include *B. pseudomallei*, and misidentifications as *Burkholderia thailandensis* have frequently occurred[4,136]. It is expected that forthcoming database updates will rectify this by including *B. pseudomallei* with spectra generated from geographically wide-ranging isolates[135]. Testing for susceptibility to ceftazidime, imipenem or meropenem, trimethoprim-sulfamethoxazole, doxycycline, and amoxycillin-clavulanate is recommended, however resistance to these agents at diagnosis (prior to therapy) is extremely rare. Epidemiological cut-off values for antimicrobials used to treat melioidosis have recently been used to develop EUCAST (European Committee on Antimicrobial Susceptibility Testing) clinical minimum inhibitory concentration and zone diameter breakpoints, which is a major advance[137]. With these breakpoints, wild-type *B. pseudomallei* is classified as 'susceptible, increased exposure (I)' to ceftazidime, trimethoprim-sulfamethoxazole, doxycycline, and amoxycillin-clavulanate, reflecting the higher doses of these antimicrobials required for melioidosis treatment compared to other indications. However, as for other drug-microorganism combinations, this new EUCAST definition of 'I' requires considerable clinician education to prevent over-use of meropenem[138]. Minimum inhibitory concentration interpretive criteria are also provided by the Clinical and Laboratory Standards Institute (CLSI)[139]. Cefiderocol is a siderophore cephalosporin with activity against Gram-negative bacteria including *B. pseudomallei*. There are no clinical breakpoints for testing *B. pseudomallei* against this new antimicrobial, however the MIC_{90} was 0.125 mg/L in a study performed in Australia, suggesting likely potential for clinical efficacy[140]. Resistance to antimicrobials due to chromosomal alterations rarely develops during treatment but can occur if there is a high bacterial burden such as in an undrained abscess, osteomyelitis, or where there is difficulty clearing pulmonary infection, as can occur in cystic fibrosis or bronchiectasis (FIG. 3).

Because it takes approximately 48 hours to isolate and identify *B. pseudomallei*, an accurate test for direct detection of *B. pseudomallei* in clinical specimens would be desirable. Indirect immunofluorescence (IIF), lateral flow immunoassays, and nucleic acid amplification tests (NAATs) with a range of targets have been developed, however to date none of them have sufficient sensitivity to replace culture assays[141-143]. Indirect immunofluorescence for detection of exopolysaccharide, and nucleic acid amplification targeting the type III secretion system are both highly specific[141,142]. Following initial problems with false positive

results, updated iterations of lateral flow immunoassays targeting the capsular polysaccharide with improved specificity on clinical specimens have been developed, and their lower cost and ease of use compared to IIF and NAAT are appealing[143-145].

A range of serological assays has been developed for melioidosis diagnosis[127]. The indirect haemagglutination assay (IHA) remains most widely used but is poorly standardised, relying on sensitisation of rabbit red blood cells to crude *B. pseudomallei* antigens. In endemic areas, particularly Southeast Asia, IHA has poor specificity due to high background seropositivity, and has poor sensitivity early in infection[146,147]. Serial IHA testing may assist with melioidosis diagnosis in returned travellers, and in culture-negative encephalomyelitis[72,127]. IHA testing is also done in the 'Top End' region of the Northern Territory in Australia as a screening test prior to immunosuppression[148].

Newer serological assays show promise but are not widely available. An enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against the O-polysaccharide has better specificity than IHA[149]. ELISA used for detection of antibodies against the haemolysin-coregulated protein (Hcp1) has been developed as an immunochromatography test, and when used in combination with antigen detection by lateral flow immunoassay, it showed a clinical sensitivity of 68% and a specificity of 95% in northeast Thailand[150]. A rapid test that can detect antibodies against Hcp1 and three additional antigens showed a reported sensitivity of 92% and a specificity of 97-100% on specimens collected in Thailand[151].

[H2] Treatment

The most important treatment advancement for melioidosis involved a randomized controlled trial in Thailand that compared ceftazidime antibiotic with the prior conventional therapy for severe melioidosis. The trial demonstrated that ceftazidime resulted in a 50% reduction in overall mortality[152]. Subsequent sequential clinical trials in Thailand and observational studies from Australia have defined the current optimal antibiotics and duration of therapy for melioidosis[153-156]. Prolonged treatment is given to prevent relapse, which occurs in approximately 4% of cases[9]. Importantly, there is limited clinical trial evidence to support the durations and dosages recommended in melioidosis treatment guidelines.

Therapy begins with an intensive phase of a minimum of 10 days of intravenous ceftazidime or a carbapenem (meropenem or imipenem), with or without addition of trimethoprim-

sulfamethoxazole (BOX 2). This is followed by an eradication phase of oral trimethoprim-sulfamethoxazole for 3 to 6 months. In very specific cases, like those involving single skin lesion without bacteremia or sepsis, an oral-only regimen of trimethoprim-sulfamethoxazole has been used. Surgical drainage of large abscesses is indicated but is usually not required for multiple small liver and splenic abscesses. Prostate abscesses usually require drainage, and this can be done under ultrasound guidance. Granulocyte colony-stimulating factor (G-CSF), a type of growth factor that stimulates the production of granulocytes and stem cells in the bone marrow, is used in some settings for cases of severe melioidosis with septic shock, although its benefits remain unclear.

[H2] Prevention

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 when exposed to direct contact with soil or water, they should only drink bottled or boiled water, and they should avoid outdoor exposure to heavy rain or dust clouds[23]. The guidelines also encourage cessation of smoking (particularly in those with underlying conditions) and discourage the application of herbal remedies or organic substances to wounds. Similar public health messaging occurs each wet season in northern Australia[18]. The International Melioidosis Network provides support for those seeking information and is a forum for sharing experiences and new findings.

In northeast Thailand, the establishment of a multifaceted community prevention program was associated with lower rates of hospital admissions for infectious diseases in general, and with lower all-cause mortality[157], but the benefits of this program in the prevention of melioidosis are not entirely clear. To improve the effectiveness of interventions, it may be necessary to modify or add behavioural change techniques and increase the frequency of the intervention.

Guidelines have been developed for post-exposure prophylaxis in selected high-risk circumstances, while noting that nosocomial and laboratory-exposure cases of melioidosis are extremely uncommon[125,153,154]. Primary prophylaxis with trimethoprim-sulfamethoxazole is used in some high-risk populations, such as patients requiring hemodialysis in Darwin, Australia during the monsoonal wet season[158].

A vaccine for melioidosis is highly desirable to reduce disease and death in at-risk populations and as defence against bioterrorism. Health economic modelling supports a cost-effective public health vaccination strategy that targets individuals in endemic regions, particularly those with risk factors such as diabetes[159]. An estimated 280 million people with diabetes live in melioidosis-endemic regions (calculated from[12,160]), representing a well-defined market for a vaccine, alongside people with kidney disease, elderly and immunocompromised individuals. However, vaccines for complex intracellular pathogens are harder to develop than predominantly blood-borne pathogens[161], and are likely to require induction of cytotoxic T cells as well as antibodies. A successful public health vaccine for melioidosis does not need to induce sterile protection, but it should boost the immune response of compromised hosts to prevent severe disease and death.

The past decade has seen an acceleration in progress towards a vaccine for melioidosis[162]. The establishment of a standardised mouse model for evaluation of vaccine candidates at the United States Army Medical Research Institute of Infectious Diseases enables accelerated selection of vaccine candidates for clinical trials. Vaccine candidates currently under development are shown in FIG. 4, with key virulence factors outlined in Supplementary Table 1. Sub-unit vaccines include those focussed on the capsular polysaccharide[163,164] – a key virulence factor – and its fragments[165], the type A O-polysaccharide of *B. pseudomallei* LPS[166], protein virulence factors like Hcp1[163], AhpC[167], OmpW[168] and other *B. pseudomallei* proteins[169]. Gold nanoparticle glycoconjugates, which act as both delivery agents and adjuvants, are being developed for a number of proteins combined with LPS[170,171]. Outer membrane vesicles (OMVs) are an exciting vaccine advance that use blebs from the bacterial membrane including multiple key immunogenic antigens[172]. Live attenuated vaccines feature *B. pseudomallei* modified for reduced virulence whilst still retaining immunogenicity[173-175].

Once a vaccine shows promising effectiveness in animal models, the next step is Phase I clinical trials. A pipeline of several potential vaccine candidates entering clinical trials in parallel is required to drive learning and progress. Good Manufacturing Practice (GMP)-compliant manufacture of candidates for human use is a major financial and logistical roadblock. A vaccine candidate based on the conserved 6-deoxyheptancapsular polysaccharide and the T6SS protein Hcp1 (CPS-CRM_{197}/Hcp1[163]) and another based on OMVs[172] are scheduled for Phase 1 clinical trials; hopefully trials for other candidates will

follow. Vaccine licensure is likely to require use of the 'animal rule' (vaccine efficacy established based on adequate and well-controlled studies in animal models of melioidosis,), with post licensing monitoring rather than the conventional randomised control trials of efficacy, due to costs and the feasibility limits of a large enough human trial. Establishment of validated correlates of protection between humans and animal models, in parallel with strengthening capacity for vaccine trials and immunology studies in highly endemic regions is therefore essential.

[H1] Conclusions and future research priorities

Melioidosis is a substantial public health concern for humans and animals in many parts of the world, with predicted increases in cases and expansion into new areas of endemicity in coming years. Support for both improved laboratory capacity in many regions and expanded field studies are required to better define the global footprint of *B. pseudomallei* and melioidosis. Further work is needed to understand the human, environmental, and climatic factors that contribute to variation in melioidosis incidence, including predictive modelling of the impact of climate change and ascertainment of timelines and mechanisms of intercontinental and regional *B. pseudomallei* dispersal. Genomic sequencing has helped to pinpoint the source of melioidosis cases, and further development and validation of methodological standards for genomic surveillance is needed.

Although epidemiological evidence suggests that host risk factors and mode of acquisition are the key determinants of disease presentation and severity, the contribution of variation in bacterial virulence genes and their expression is not known. Bacterial genome-wide association studies and host-pathogen transcriptomics could provide further insight into the factors contributing to the clinical heterogeneity of melioidosis.

Access to laboratory diagnostics is currently a barrier to diagnosing melioidosis in many regions, delaying initiation of timely therapy and contributing to the incomplete understanding of the global burden of the disease. Lateral flow immunoassays have been an exciting development, and further refinement and validation of these and new diagnostic point-of-care technologies is a priority. Fine-tuning of treatment guidelines and access to intensive care, have substantially improved outcomes. However, there are remaining questions regarding optimal melioidosis treatment, which could be addressed with multi-centre clinical trials. For example, it could be tested whether oral eradication therapy is

always needed (particularly if prolonged intravenous treatment is given), and whether some presentations in addition to skin infection with a single lesion could be managed with oral therapy alone. While public health campaigns in endemic areas target individuals at risk of melioidosis, it is likely that barriers exist to enacting recommended protective measures; treatment and prevention of melioidosis risk factors such as diabetes, hazardous alcohol consumption, and chronic kidney disease are priorities. Chemoprophylaxis is given to some at-risk individuals in northern Australia, however its optimum use, including the balance with potentially severe trimethoprim-sulfamethoxazole adverse effects, has not been defined and requires further assessment. There are several vaccine candidates which may attenuate disease severity in individuals with risk factors; these are ready for phase 1 clinical trials. Further work is needed to understand the immune correlates of severe disease and death.

While substantial progress has been made in our understanding of the epidemiology, pathophysiology, and optimal methods and approaches for diagnosis, treatment, and prevention of melioidosis, there remains much more to learn. Meanwhile the large disparity in melioidosis mortality between affluent countries and many melioidosis-endemic regions largely reflects issues of access to laboratories with diagnostic capacity, appropriate antimicrobial therapies, and state-of-the-art intensive care management.

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Author contributions

All authors contributed equally to the article.

Competing interests

The authors have no competing interests to declare.

Related links

International Melioidosis Network:

https://www.melioidosis.info/infobox.aspx?pageID=101

Supplementary information

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Figures

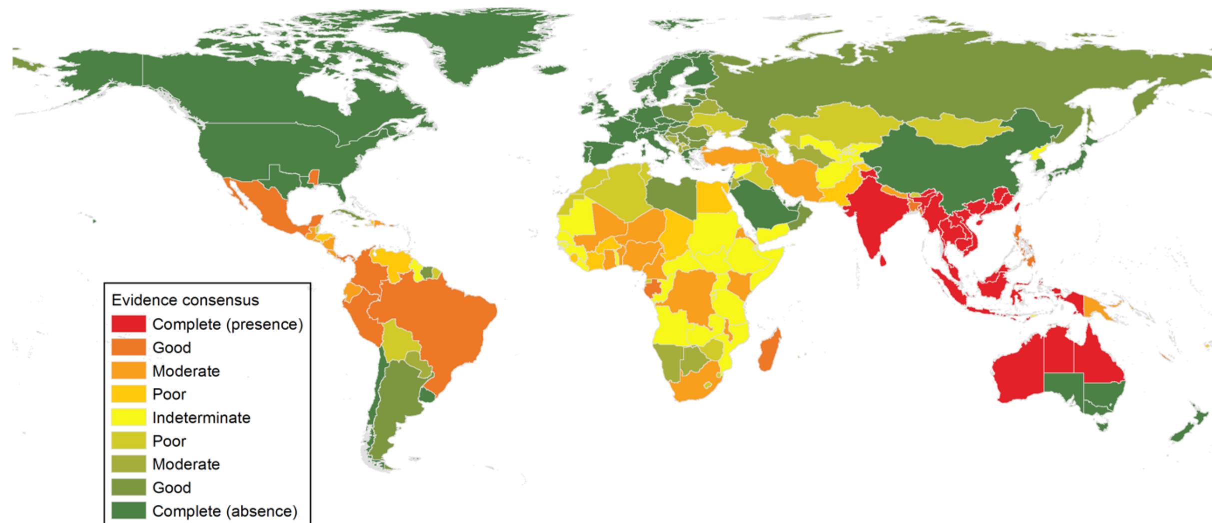


Fig. 1. Global distribution of *Burkholderia pseudomallei*. The map represents the global distribution of *B. pseudomallei*, based on consensus evidence gathered from January 1910 to September 2022. Green colour represents a complete consensus on absence of *B. pseudomallei* and red a complete consensus on presence of *B. pseudomallei*. To obtain updated global consensus evidence and perform this analysis we used a weighted scoring system with a method modified from a previous modelling study[12] (Supplementary Information).

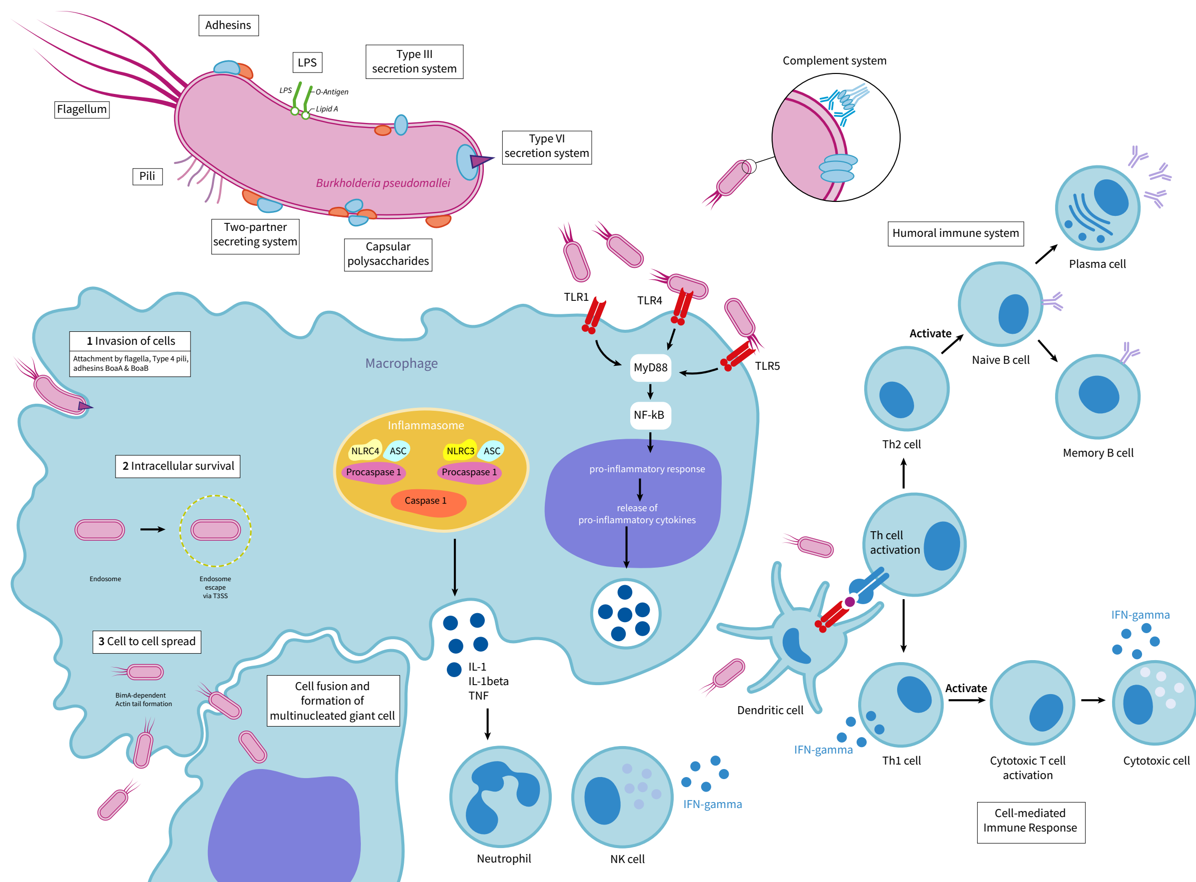


Fig. 2. Virulence factors of *Burkholderia pseudomallei* and the host immune response. a) *B. pseudomallei* putative virulence factors include the lipopolysaccharide (LPS), the type III secretion system (T3SS), the type VI secretion system (T6SS), the two-partner secreting system, the capsular polysaccharide, the flagellum, pili and adhesins. b) *B. pseudomallei* can invade macrophages by attaching to the cell surface using flagella, the type IV pili and BoaA and BoaB adhesins and injecting effector proteins through the T3SS. After invasion, *B. pseudomallei* can persist and reproduce within the cell for extended durations. After internalisation *B. pseudomallei* can escape from endocytic vacuoles into the cytoplasm using its T3SS by lysing the endosome membrane. Thereafter, *B. pseudomallei* can spread to neighbouring cells through BimA-dependent actin-based membrane protrusions and form multinucleated giant cells by cell fusion. c) Pattern recognition receptors (PRRs), such as membrane bound Toll-like receptors (TLR)-1, TLR4 and TLR5 will first detect *B. pseudomallei* and initiate the immune response via activation of the nuclear factor-κB (NF-kB) pathway. In concert, the intracellular inflammasomes NLRC4 and NLRC3 mediate release of interleukin (IL)-1 and IL-18, inducing protective interferon (IFN)-γ production and recruitment and activation of neutrophils and natural killer (NK) cells. Tumor necrosis (TNF) and IL-6 release

will further activate the complement and coagulation systems. Activation of dendritic cells will lead to Th cell activation which will result in a cell-mediated immune response by cytotoxic T cell activation, or a humoral immune response via Th2 cell proliferation, with the resulting production of specific antibodies. MyD88: myeloid differentiation factor 88; ASC: apoptosis-associated speck-like protein containing a caspase recruitment domain[1,86,176].

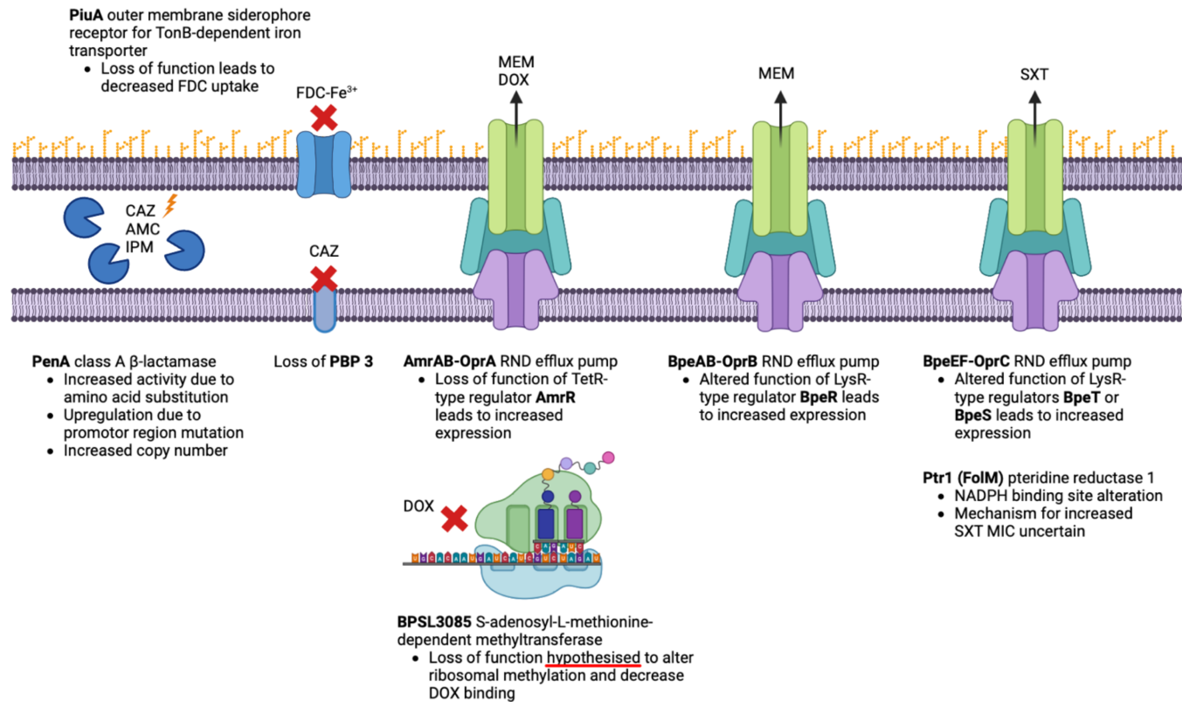


Fig. 3. Antimicrobial resistance mechanisms of *Burkholderia pseudomallei*. The chromosomal changes leading to the shown antimicrobial resistance mechanisms can occur and be selected for during treatment but are very rare. Upregulation, alteration, or copy number variation of the PenA class A β-lactamase can lead to ceftazidime, amoxicillin-clavulanate, and/or imipenem resistance[89,177-183]. Ceftazidime resistance has been reported in association with loss of penicillin binding protein 3 (PBP-3)[184]. Upregulation of resistance-nodulation-division (RND) efflux pumps (AmrAB-OprA, BpeAB-OprB, BpeEF-OprC) is associated with elevated meropenem, doxycycline, and/or trimethoprim-sulfamethoxazole minimum inhibitory concentrations[89,181,185,186]. Altered ribosomal methylation due to a loss-of-function mutation in the S-adenosyl-L-methionine-dependent methyltransferase (encoded by *BPSL3085* gene) is a hypothesised mechanism of doxycycline resistance[89,182,187,188]. Folate pathway mutations including alteration of Ptr1 (FolM) pteridine reductase 1 can lead to trimethoprim-sulfamethoxazole resistance, however the mechanisms are incompletely understood[89,181,185,186]. Loss of function of the TonB-dependent iron transport receptor PiuA is associated with cefiderocol resistance[189]. Abbreviations: Ceftazidime, CAZ; meropenem, MEM; imipenem, IPM; trimethoprim-sulfamethoxazole, SXT; doxycycline, DOX; amoxycillin-clavulanate, AMC; cefiderocol, FDC;

minimum inhibitory concentration, MIC; nicotinamide adenine dinucleotide phosphate, NADPH.

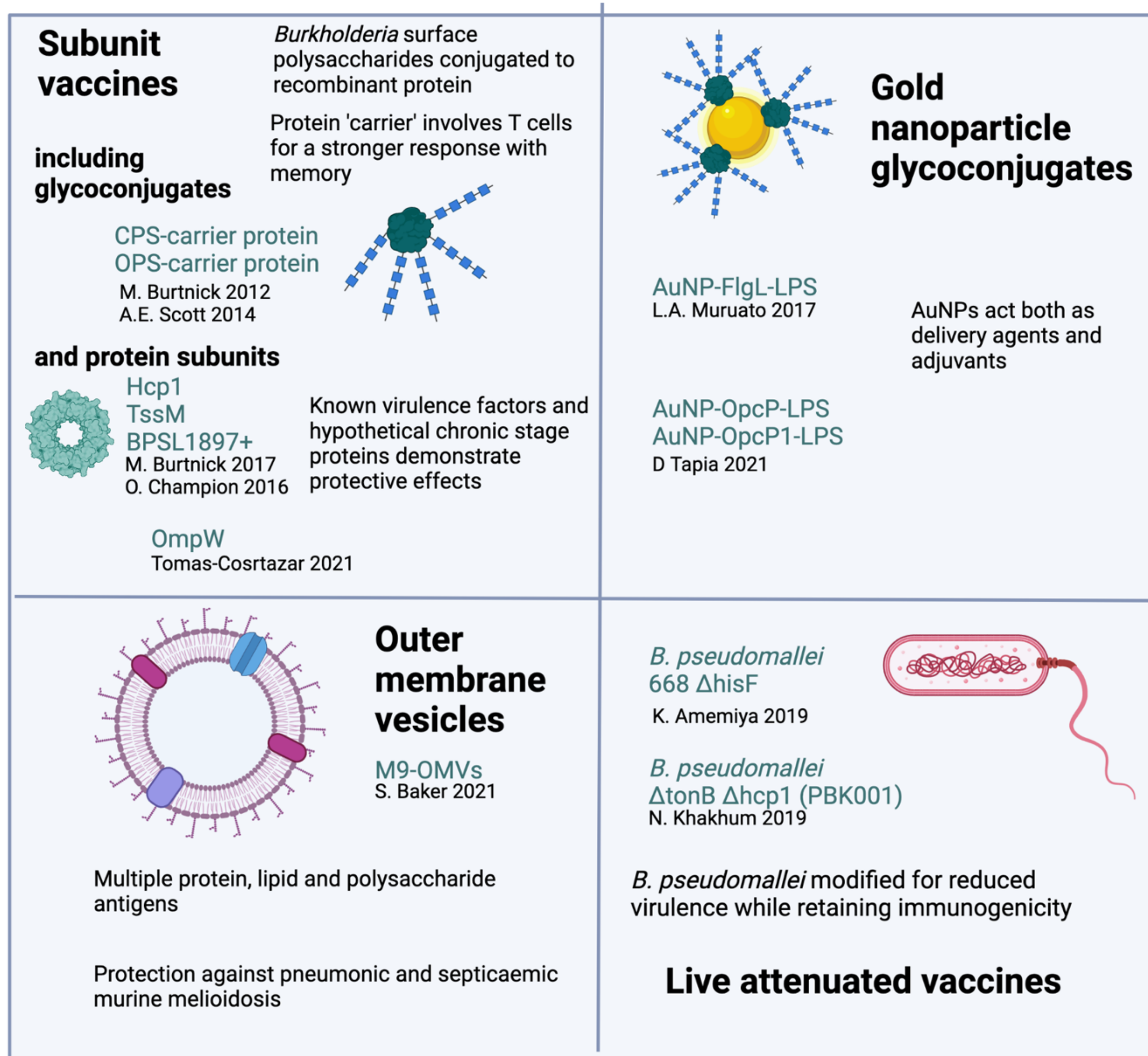


Fig. 4. Vaccines against *Burkholderia pseudomallei* currently under development. Schematic overview of different approaches to develop a vaccine for melioidosis. a) Subunit vaccines can be developed using *B. pseudomallei* surface polysaccharides conjugated to a recombinant protein including CPS-carrier protein[164] and OPS-carrier protein[165] and to target protein subunits from the bacteria, for example, Hcp1 and TSmM[163], BPSL1897+[169] and OmpW[168]. b) Outer membrane vesicles (OMVs) can carry multiple proteins, lipid and polysaccharide antigens and confer protection against pneumonic and septicaemic murine melioidosis. An example is the M9-OMV vaccine[172]. c) Gold nanoparticle (AuNP) glycoconjugates constitute another vaccine strategy, where AuNPs act both as delivery agents and as adjuvants. Some examples include AuNP—FlgL-LPS[170] and AuNP-OpcP-LPSn[171] d) Live attenuated vaccines are developed from *B. pseudomallei* cells that are modified to have reduced virulence while still retaining immunogenicity. Some examples of such

vaccines are 668 Δ*hisF*[173] and Δ*tonB* Δ*hcp1* (PBK001) Δ[174]. For a more comprehensive overview of vaccines for melioidosis, see ref.[162].

BOX 1. Protective immunity against melioidosis.

Around 53 to 84% of individuals with melioidosis admitted to hospital have at least one risk factor affecting their immune system, such as diabetes, renal disease, alcohol excess or older age[9,29]. This tells us that a healthy immune system usually prevents invasive disease, unless there is a huge bioburden, as occurs during near-drowning incidents in contaminated water or potentially inhalational melioidosis resulting from the intentional release of *Burkholderia pseudomallei*.

Early control of *B. pseudomallei* by the innate immune system is likely to be important, as shown by the association between pattern recognition receptors such as Toll-like receptors and survival[92-95,190]. Neutrophils play a critical defence role in mouse models[101], and people with diabetes show impairment in phagocytosis and neutrophil migration in response to *B. pseudomallei* infection[191]. Antibodies against the bacteria as measured by indirect haemagglutination assay (IHA) in human populations may be a marker of exposure to *Burkholderia* species in the environment rather than protection[24,146,147]. However, murine studies have demonstrated protection to be antibody dependent[174] or at least conferred by passive transfer of antibodies against bacterial polysaccharides[192,193]. Associations have been reported between survival from melioidosis in humans and antibody levels[123,146,194,195], including in the immunoglobulin G2 (IgG2)[122] and immunoglobulin G3 (IgG3)[123] subclasses, as well as a role for functional antibodies[196,197]. Cellular immune responses against this intracellular pathogen are important for host survival, with mouse studies showing the role of interferon-gamma (IFN-γ)[198], T cells and natural killer (NK) cells[120,121]. In humans, increased risk of disease[199] and death[118] in certain HLA haplotypes is evidence of a central role for T cells. T cell responses to *B. pseudomallei*[114-116,200] and individual proteins[117-119] are higher in survivors compared to fatal cases. Human immunodeficiency virus (HIV) is not a major risk factor for melioidosis[201], suggesting that $CD4^+$ T cells are not the key driver of protection, such that $CD8^+$-mediated cytotoxicity for infected cells may be more important[115,116]. Survival from melioidosis is also associated with elevated levels of NK cells

and expression of the chemokine receptor CX3CR1, and patients with diabetes use different pathways for survival, including higher antibody production against *B. pseudomallei* and gamma-delta (γδ) T cells[116].

Overall, protective immunity against melioidosis is likely to utilise a pattern of responses including neutrophils, NK cells, antibodies and T cells. Successful vaccination strategies will stimulate responses across immune compartments to boost the immune systems of vulnerable people and confer protection against potential biodefence threats.

Supplementary Information

Development of melioidosis evidence consensus database at national level for generation of Fig. 1

The new global map (Fig. 1) includes evidence of melioidosis in 12 new countries; including Benin[202], Cameroon[203], Democratic Republic of Congo[204], Eritrea[205], Federal States of Micronesia[206], Ghana[35,207], Mali[208], Nepal[209-212], Nicaragua[213], St Kitts and Nevis[214], Trinidad and Tobago[215] and most recently the southern United States[216,36].

We used a weighted scoring system to update global evidence consensus using the method modified from a previous modelling study[217] (Supplementary Fig. S1).

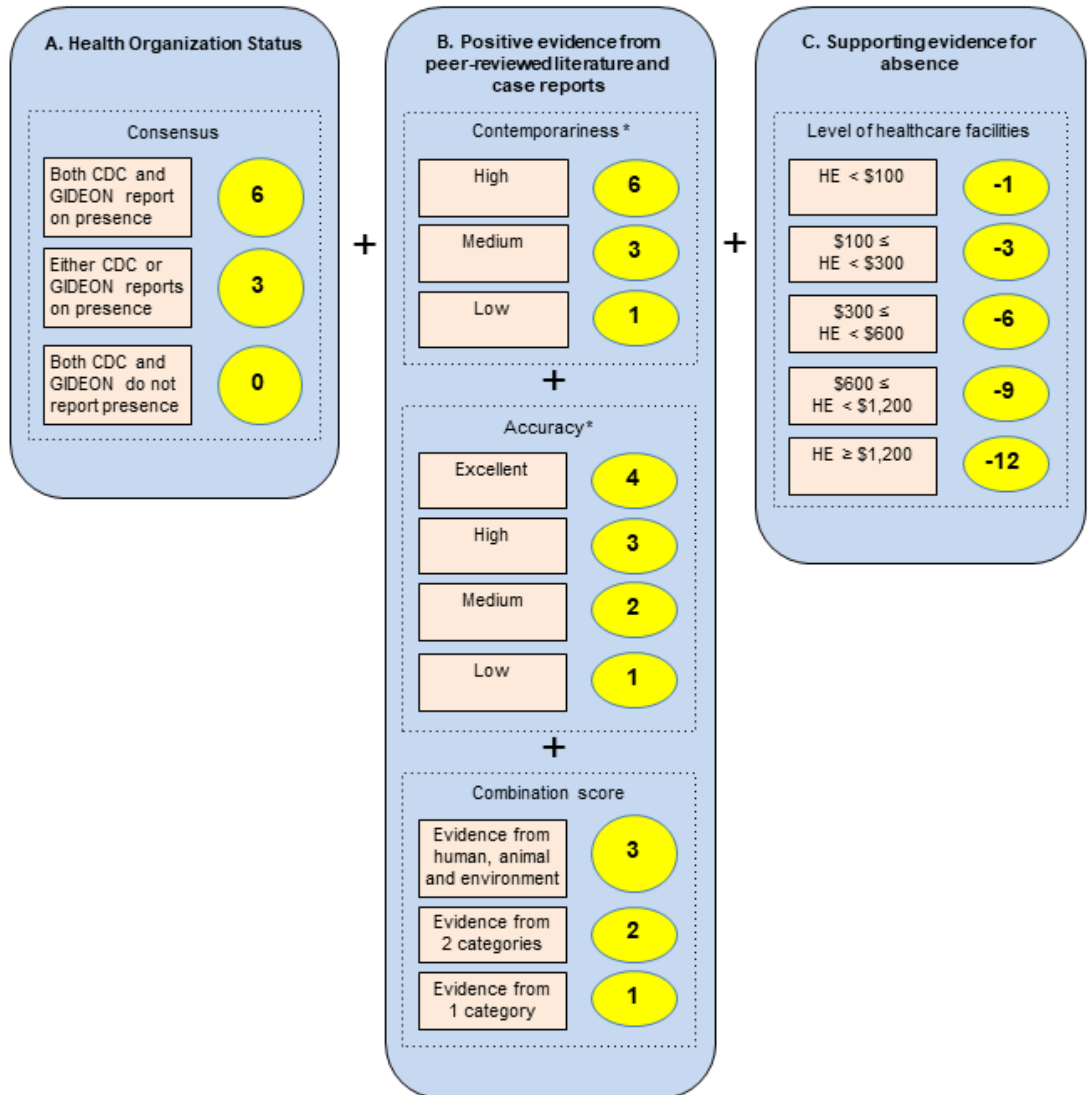
For health organization evidence, we used the data from two health reporting organizations (Centers for Disease Control and Prevention (CDC) and the Global Infectious Disease and Epidemiological Network (GIDEON)). The World Health Organization (WHO) does not provide data at country/territory level. A consensus of presence or absence (++ or --) scored six or zero, respectively, while a lack of consensus (+- or -+) scored three. This gave a maximum score for this category of six.

For supporting evidence of positive occurrences, each publication and case report about melioidosis cases or presence of *B. pseudomallei* with a definite bacterial identification by either genotyping, PCR identification, latex agglutination, animal virulence test or arabinose test (for environmental *B. pseudomallei*) was scored independently for contemporariness and diagnostic accuracy. For contemporariness, the year of the last occurrence was used for scoring as follows: between 2013-2022-2013=6, 2003-2012=3, pre 2003=1. This score was then added to a score for accuracy, whereby excellent accuracy and a score of four was characterised by a report of more than ten indigenous culture-confirmed cases at a single location, or more than ten environmental samples culture-positives for *B. pseudomallei* at a single location. High accuracy and a score of three was characterised by reports of more than five indigenous culture-confirmed cases at a single location, or more than five environmental samples culture-positives for *B. pseudomallei* at a single location. Medium accuracy and a score of two was characterised by a report of indigenous culture-confirmed case or presence of *B. pseudomallei* from the environment. Low accuracy and a score of one

was characterised by a report of an exported case. To provide a single score per country/territory, the highest-scoring per country/territory was calculated. To represent the diversity of evidence, evidence was categorized into three types; human cases, animal cases and environmental detection. If a single type of evidence was present in a country/territory, a score of one was added. If two types were present, a score of two was added. If all types were present, a score of three was added. This resulted in a maximum available score of 13 for this category.

For supporting evidence of absence, the evidence was graded using total annual healthcare expenditure (HE) per capita at average US Dollar exchange for the year 2019. Higher HE has been linked to better overall public health infrastructure, which includes high-quality diagnostic resources. Therefore, the lower the HE, the less certain we can be that an absence of data accurately reflects an absence of cases. All overseas territories were assumed to have the same HE as their parent nations. The following criteria were used. For countries/territories with no evidence of positive occurrences, having HE < $100 gave a score of -1, $100 ≤ HE < $300 gave a score of -3, $300 ≤ HE < $600 gave a score of -6, $600 ≤ HE < $1,200 gave a score of -8 and HE ≥ $1,200 gave a score of -12. The maximum score for this category was -12.

We derived an overall country/territory evidence score by adding the scores for all three evidence categories, dividing by the maximum possible score (19 when score was higher than or equal to zero, and 12 when score was less than zero) and then multiplying by 100. Evidence consensus was then categorized into nine interval categories from 100% to -100%, defined as complete (±80% to ±100%), good (±60% to ±80%), moderate (±40% to ±60%), poor (±20 to ±40%) or indeterminate (-20 to 20%).



Supplementary Fig. S1. Overview of the evidence scoring system. Dashed lines surrounding individual parameters that were assessed and totaled in the scoring system. Evidence consensus was calculated as the proportion of the maximum possible score. For contemporariness, the year of occurrence between 2013-2022 was classified as high, 2003-2012 as medium, and pre-2003 as low. For accuracy, excellent accuracy and a score of four was characterised by a report of more than ten indigenous culture-confirmed cases at a single location, or more than ten environmental specimens culture-positives for *B.*

pseudomallei at a single location. High accuracy was characterised by a report of six to ten indigenous culture-confirmed case or six to ten environmental specimens culture-positives for *B. pseudomallei* at a single location. Medium accuracy was characterised by a report of one to five indigenous culture-confirmed case or one to five environmental specimens culture-positives for *B. pseudomallei* at a single location. Low accuracy was characterised by a report of an exported case. HE = Total healthcare expenditure per capita at average US $ exchange rates. * Each individual piece of peer-reviewed evidence was scored for contemporariness and accuracy before taking the average of the whole set then adding to the combination score.

Supplementary Table S1. Virulence factors of *B. pseudomallei* – updated from Wiersinga *et al*. 2017[1]

Virulence Factor	General Role	Description	Ref.
Flagella	Adherence	Adhesion, motility, inflammation.	218,219
PilA		Adhesion factor and intracellular mobility. PilA mediates temperature dependent adherence and formation of microcolonies in some *B. pseudomallei* strains and gene deletion showed reduced killing in BALB/c murine model.	220,221
Boa/BoaB		T5SS autotransporters. Cell attachment, adhesin, autotransporter and possible role in intracellular replication.	222-224
OmpW		Outer membrane protein W plays role in adherence.	225
IrlR	Invasion	Two component response regulator, reduced invasion in mutants.	226
BopE		T3SS, BopE is a guanine nucleotide exchange factor targeting Cdc42 and Rac1, inducing actin rearrangements (aiding invasion).	227-229
BopA		Involved in avoidance of autophagy, phagosome membrane disruption.	228,229
BipD	Endocyte escape	T3SS effector proteins, phagolysosome survival and escape, cell invasion.	227,230,231
BsaQ		T3SS structural protein. Involved in phagosomal escape, cell invasion and plaque formation.	232,233
BsaZ		T3SS structural protein. Delayed vacuolar escape, limited replication and MNGC formation.	230,234
BsaU		T3SS. Involved in early onset activation of caspase-1 pathway in macrophages, delayed escape.	235
CHBP (cif homolog)		T3SS effector. ATP/GTP binding protein that delays host cell maturation, arresting cycle in G2/M and impeding apoptosis.	231,236-239
PurM/PurN	Intracellular survival	Phosphoribosylaminoimidazole formyltransferase/synthetase purine biosynthetic pathway, decreases replication.	235
SodC		Superoxide dismutase and other enzymes (KatG, AhpC and DNA binding protein DpsA) mediate resistance to oxidative stress.	240-243
AhpC		The alkyl hydroperoxide reductase subunit C protects against host oxidative stress.	117
RpoS		Internalisation and macrophage fusion. Suppress iNOS by upregulating SOCS3 and CIS cytokines.	244,245
BipB		Involved in vacuolar escape, MNGC formation and reduced cytotoxicity.	234,236,246

Virulence Factor	General Role	Description	Ref.
BimA	Actin based motility	T5SS effector. Escape from phagosome, autotransporter, actin tail formation. Encephalomyelitis strongly correlated with $BimA_{Bm}$ allele strains in Australian cohort.	71,247
Hcp1	Others	Tail spike T6SS-1, MNGC cell formation, macrophage cytotoxicity. Induces IL-10 and TGF-β.	248-251
VirAG		T6SS regulators, sensor and histidine kinase. Host cell fusion.	64,238
TssM		T2SS (part of Gsp) effector. Deubiquitinase targets TRAF3, TRAF6 and IκBα to inhibit type I IFN and NFκB pathways.	252,253
CPS		Four CPS structures, CPSI-IV. Protects from C3b complement and NHS, CPSIII has environmental role. Biofilm production, not essential for survival but contributes towards persistent infection/latency.	254-256
LPS		3 serotypes, smooth type A predominates, confers resistance to NHS and from cationic peptides. Reduced minimal pyrogenic lethal toxicity and macrophage activation. Length, number and position of fatty acyl chains can affect LPS bioactivity and has recently been shown to vary between virulent strains.	111,257-263
Acyl-homoserine lactone (AHL) quorum-sensing (QS)		Mediated by AHLs and a second system using HMAQ. Upregulates transcription of genes simultaneously within a population involved in colonisation, longer survival and higher LD_{50}.	264,265
RpoE		Biofilm formation, heat stress response via RpoH regulated heat-shock proteins, oxidative and osmotic stress. Mutants show reduced intracellular survival in macrophages.	266
BLF1 (*Burkholderia* lethal factor-1)		Similar to *E.coli* cytotoxic necrotising factor. Irreversibly interferes with initiation of translation by inactivating eIF4A and thereby recruitment of 40S ribosomal subunit thus protein synthesis. Cell cytoskeleton alteration and cell death. Concentrations low as 2.5x10-7M can cause effect with molecular turnover like that of ricin.	267,268
Morphotype switching		Seven morphotypes- wrinkled type 1 predominates. Strain differences in colony morphology phenotypically lead to changes in biofilm production, secreted enzymes and motility, hence influencing intracellular survival, lethality and persistence.	269

Abbreviations: Cdc42, cell division control protein 42 homolog; Rac1, Ras-related C3 botulinum toxin substrate 1; MNGC, multi-nucleated giant cell; iNOS, inducible nitric oxide synthase; SOCS3, suppressor of cytokine signaling 3; CIS, cytokine inducible src homology 2 containing protein; Gsp, general secretory pathway; NHS, normal human serum; CPS, capsular polysaccharide; LPS, lipopolysaccharide. This Table is an updated version of the one published in Wiersinga WJ, Virk HS, Torres AG, et al. Melioidosis. Nat Rev Dis Primers 2018;4:17107.

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