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# A REVIEW ON MELIOIDOSIS

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## ABSTRACT

Melioidosis is a fatal disease caused by *Burkholderia pseudomallei* and the disease is endemic in Southeast Asia and northern Australia. This organism is resistant to most of the antibiotics and there is a lack of an effective vaccine for this disease. Thus, there has been a recent resurgence of interest in the disease and there has not yet been the definite study that describes to solve these aspects of the disease. The awareness is required among the clinicians and laboratories, isolating and identifying the organism. Thus, the preliminary studies on the disease are required to design the new mode and reliable treatment for melioidosis. This review covers the fundamental aspects of the disease and its causative agent which could be helpful for researchers.

**KEYWORDS:** **Melioidosis, *Burkholderia pseudomallei,* pathogenesis, diagnosis, treatment.**

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# INTRODUCTION

Melioidosis is a disease caused by soil saprophyte organism, *Burkholderia pseudomallei*. The disease is endemic in Southeast Asia and northern Australia and it was described earlier as glanders like disease. In 1911, the organism was first isolated from a young boy who was dying with pneumonia by a British pathologist Alfred Whitmore at Rangoon General Hospital, Burma. The term melioidosis was derived from two Greek words: 'melis' (distemper of asses) and 'eidos' (resemblance) and it was coined by Stanton and Fletcher in 1921. In endemic tropical regions, melioidosis is now recognized as a major cause of fatal septicaemia [1]. Usually, the disease is characterized by pneumonia and occasionally by multi organ abscesses in the patient with defined risk factors and with mortality rate of up to 40% [2].

## *CAUSATIVE AGENT*

The *Burkholderia pseudomallei* was initially classified under the species *Pseudomonas* as it exhibits similar culture and morphological characteristics and biochemical properties [3]. Based on the 16S ribosomal RNA sequences, DNA-DNA homology values, cellular lipid and fatty acid composition, and phenotypic characteristics, seven species in *Pseudomonas* genus were transferred to the new genus *Burkholderia* [4]. The bacterium has tendency to cause the disease after inhalation and this characteristic makes the pathogen to include in the Centers for Disease Control (CDC) list as a category B agent [5]. The *Burkholderia pseudomallei* is an environmental Gram-negative bacilli, motile [1], facultative intracellular bacterium [6].

## *GENOME PLASTICITY*

The genome of *Burkholderia pseudomallei* composed of two chromosomes of 4.07 megabase pairs and 3.17 megabase pairs and encodes 3,460 and 2,395 coding sequences respectively. The large chromosome carries the core functions associated with the metabolism and cell growth where as the small chromosome carries most of the accessory functions associated with adaption and survival in different niches. The two chromosomes share a very little similarity except in rRNA clusters. The organism contains 42 insertion sequence elements and 26 pseudogenes. One of the most striking features of the genome was the presence of 16 genomic islands that constitute 6.1% of the genome [5]. The comparative analysis of *B. pseudomallei* proteins with *Ralstonia solanacearum* produced highest number of orthologue matches whereas with more distant pseudomonads, produced fewer matches [7].

## *VIRULENCE FACTORS OF BACTERIA*

*B. pseudomallei* is a flexible bacterium which makes it to survive in wide variety of antagonist environment, including nutrient deficiency, acid and alkali pH, disinfectant and antiseptic solutions, exposure to many antibiotics, and extremes of temperature. It adapts to its many hosts, producing proteases, lipases, lecithinase, catalase, peroxidase, superoxide dismutase, hemolysins, a cytotoxic exolipid, and a siderophore. They are resistant to complement lysosomal defensins and cationic peptidases [8]. It produces an important virulence determinant; glycocalyx polysaccharide capsule [9]. There is a phenotypic change in the organism and results in significant antibiotic resistance when the capsule allows forming microcolonies in a protective environment [10]. The organism alters the external surfaces of the infected cells which causes the surfaces to fuse with the membranes of neighboring cells leading to Multi Nucleated Giant Cell (MNGC) formation and actin-associated membrane protrusion. This contributes cell to cell spreading in infected hosts [6].

## *PATHOGENESIS*

Melioidosis predominantly affects the people who are in regular contact with soil and water. The organism gains entry through the abrasion of existing wounds or by the aspiration of contaminated water during near-drowning episodes or iatrogenic inoculation and laboratory acquired infection. Among

these, the most common mode of infection is inoculation [1, 3]. The disease usually presents with a broad clinical spectrum ranging from mild localized infection to rapidly fatal septicemia [11]. The organism has the tendency to remain latent in the host for long periods of time and when conditions become favorable for the bacterium to multiply, the disease may recrudesce clinically [12-14]. In endemic areas, a significant proportion of apparently healthy individuals have antibodies to *B. pseudomallei* which may result from subclinical infection [15]. The previous studies have demonstrated contradictory results in the role of phagocytes in melioidosis. It has been proved that the organism can survive and multiply within professional phagocytes including macrophages or monocyte and neutrophil cell lines [16, 17]. The organism has the ability to evade phagosome-lysosome fusion and destroy the phagosome membrane as soon as 15 min after ingestion [18]. In ultra structural studies of the interaction between macrophage and *B. pseudomallei*, the responses have compared between the patients with melioidosis and healthy controls and it suggests that there is less early phagolysosome fusion in macrophages from melioidosis patients, resulting in higher intracellular bacterial concentrations [1].

TNF-α is an early and a potent proinflammatory cytokine which is primarily produced by macrophages but also by B cells, T cells and fibroblasts. The increase in the level of TNF-α is associated with mortality. However, the infection can be suppressed by TNF-α and in a mouse model, the neutralization of TNF-α shows increased susceptibility to melioidosis [19]. Another study shows that the interferon-γ plays a vital role in controlling melioidosis and they suggest that the rapid production of IFN- γ, occurring in the innate response to *B. pseudomallei,* can be achieved not only by the natural-killer cells, but also by the CD8+ cells which are activated by a cytokine-dependent bystander mechanism [20]. The studies have been proved that the individuals develop infection when they fail to mount an adequate cell mediated immunity response where as the individuals who develop strong specific cell mediated immunity response to organism may not develop clinical disease [15].

## *RISK FACTORS*

The major risk factors associated with development of melioidosis includes diabetes, chronic kidney disease, chronic liver disease, connective tissue disease requiring steroid therapy or other immunosuppressive treatment, alcoholism, malignancy and traumatic injury [21]. A case control study conducted in four hospitals in northeastern Thailand confirms that diabetes is the important risk factor for melioidosis. An *in vivo* and *in vitro* study proved that the insulin markedly inhibits the growth of *B. pseudomallei* in the melioidosis patients with diabetes [22]. The disease mainly affects rice farmers and their families. The patients with thalassemia are at higher risk of developing melioidosis. The preexisting renal diseases can be an independent risk factor for melioidosis [23]. Further analysis of risk factors commonly observed in melioidosis patients demonstrates that these are all complex diseases associated with a number of compilations, increased risk for infection and immunosuppression.

## *CLINICAL DESCRIPTION*

The time taken for *Burkholderia pseudomallei* to develop the clinical onset after exposure is highly variable and it is very hard to define. A prospective study shows that the incubation period was 1-21 days (average 9 days) in 25 % of cases who had the percutaneous exposure to the soil during the monsoon [24]. The disease can be seen as bacterial pneumonia, septicaemia, acute localized infection of skin and soft tissues following trauma, inoculation, chronic infection or even acute cholangitis. The organism is usually spread by direct skin contact through ingestion or inhalation with contaminated source and it is not spread from person to person [25]. An acute septicaemic infection is the most severe form of active infection and it has a high mortality rate. A study reported that it presents with pyrexia of unknown origin (PUO) or visceral abscesses, or septic arthritis [26]. Histopathologically, the disease is characterized by abscess formation in a

variety of organs and associated with necrotizing fasciitis and sepsis. Cutaneous abcesses may be localized to skin or it may be result from bacteremic spread to the skin [27]. The localized cutaneous abscesses occur without systemic infection occasionally. A case report of isolated cutaneous melioidosis breast abscesses in a young adult suggests that melioidosis may be a more common cause of cutaneous abscesses in Western Hemisphere [28]. The polyarteritis nodosa, severe skin pustules with sepsis and extensive ecthyme-like lesions with disseminated melioidosis are the cutaneous manifestations of melioidosis. Leukocytosis, lymphopenia and hypoalbuminemia can be the markers of severity of this type of infection [29]. In case of neurological melioidosis, the most prominent feature include unilateral limb weakness, cerebellar ataxia and paraparesis [30] where as acute pulmonary melioidosis is characterized by high fever and pulmonary distress which is followed by the appearance of abscesses and death occur within a few days if the patient is untreated [31].

## *EPIDEMIOLOGY*

Melioidosis recognize within Australia from an outbreak in sheep in 1949 in Winton, northern Queensland [32]. In the Top End of the Northern Territory, the annual incidence was 16.5 per 100,000 between 1989 and 1999 [33] and after ten years, the incidence was increased to 41.7 per 100,000 as there was high annual rainfall [34]. The infection is rare in the Port Moresby region [35] and it is the emerging disease in Western Province [36]. The previous studies show that the disease is also prevalent in Malaysia, Thailand, Singapore, Indochina and Indonesia [32] and some authors consider that China and Korea is melioidosis endemic areas [37]. Although melioidosis is mostly endemic in the tropical region, occasional cases have arisen in subtropical and even temperate climates. In 1991, the incidence of melioidosis was reported in India and till 2009, 92 patients from the Indian states like Maharashtra, Kerala, Orissa, Tripura, Tamil Nadu, West Bengal and Assam [38-41] were diagnosed to have the disease and the diagnosis was performed in the southern coastal regions [42].

## *LABORATORY DIAGNOSIS*

The clinicians can suspect for melioidosis when the patients present with fever along with the history of travelling to endemic areas where melioidosis is predominant or staying there; any contact with soil or water that contain *Burkholderia pseudomallei*; and the presence of risk factors such as diabetes mellitus, kidney or liver disease [43]. The current gold standard for definitive diagnosis of melioidosis is bacterial identification by culture from the specimens such as blood, sputum and other clinical samples. This method has several drawbacks like, it requires enrichment followed by several days of incubation and this result in the delay of administering antibiotics to the patients [44]; and moreover it is difficult to identify the organism from the culture contaminants and it developed resistant to most of the antibiotic used in the empirical therapy [45]. Therefore, in order to speed up the diagnosis, various nucleic acid and antigen detection tests and serology assays can be performed which provide the results rapidly when compared to the classical culturing method. The sensitivities of both direct immunofluorescent technique and rapid immunofluorescent technique were 66 % and specificities were 99.4 % and 99.5 % respectively [46]. The latter afford the specific results in 10 min [46] whereas the former provides in 2 hours [47]. A study reported that the indirect immunofluorescent antibody test may be useful for the rapid diagnosis of the disease and their results shows that the negative predictive value of IgM assay was 92 % whereas the positive predictive value was 100 % when both IgM and IgG consider together [48].

The indirect haemagglutination assay (IHA) is widely used but it lacks sensitivity. A study has been shown that approximately half of the patients diagnose to have the disease by culture method but it was not detected by IHA [49]. This directs to the development of other serology assay like enzyme linked immunosorbent assay (ELISA) and immuno Polymerase Chain Reaction (PCR). These assays can detect the antibodies of *Burkholderia pseudomallei* [50]. The Immunochromatographic test (ICT) IgG kits can be employed to diagnose melioidosis in

the patients who travel the endemic areas of melioidosis [45]. Therefore, the diagnosis can be made at the earliest by using both culture method with one of the above mentioned serological assays as the condition of patient becomes worsen when there is delay in diagnosis after the clinical presentation.

### *TREATMENT*

Antimicrobial resistance is now a global issue [51]. *Burkholderia pseudomallei* is resistant to penicillin and its derivatives, first and second generation cephalosporins and it is susceptible to some third generation cephalosporins (remarkably ceftazidime, but also cefotaxime and ceftriaxone) [52, 53], carabapenem and fluroquinolones [52, 54], amoxicillin – clavulanate and co-trimaxazole (a combination of trimethoprime-sulfamethoxale (TMP_SMX)) [55]. The current drug of choice used to treat melioidosis is Ceftazidime [26] and it can be infused intravenously with or without trimethoprime-sulfamethoxale (TMP-SMX). It is then followed by maintenance therapy which includes four-drug regimen of chloramphenicol, doxycycline and TMP-SMX. The carbapenems are used when the patients are allergic or not responding to first line regimen [56]. And also, a study has been proved that the anti microbial peptide, cathelicidin has the bactericidal activity against *Burkholderia pseudomallei.* Hence, cathelicidin is an alternative therapeutics in the treatment [57]. As the risk factors of infection are related to the functional neutrophil defects, granulocyte colony-stimulating factor (GCSF) can be employed for the melioidodsis patient with septic shock [58].

## CONCLUSION

Melioidosis is a devastating disease of public health importance mostly in tropical areas. The causative agent can be considering as an opportunistic pathogen that may increase the mortality rate among the healthy individuals if it is not diagnosed early or the lack of appropriate antibiotic treatment. The research on preventive measures, earlier clinical identification and better management of the disease should be carried out which will reduce the burden of this disease.

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