

EMERGING PATHOGEN *BURKHOLDERIA PSEUDOMALLEI*: WHAT DO WE KNOW

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ABSTRACT:

Melioidosis, also called Whitmore's disease, is an infectious disease caused by the bacterium *Burkholderia pseudomallei*. It is reported from tropical countries, especially in Southeast Asia and northern Australia where it is widespread. The bacterium is found mainly in water and soil. Humans and animals acquire the infection by inhalation, ingestion and contact with surface water and contaminated soil, especially through skin abrasions. Melioidosis has protean clinical manifestations from acute febrile illness, localised acute or chronic suppurative infections to fatal septicaemia. However, it is important to note that melioidosis has a wide range of signs and symptoms that can be mistaken for other diseases such as tuberculosis or more common forms of pneumonia. Melioidosis which was once considered exotic in our country has been reported frequently from many areas especially from coastal areas. In some cases it is missed due to ignorance of the bacteria among microbiologists and treating physicians. Creating awareness about the disease is the need of the hour. This is very important due to the fact that, following diagnosis of melioidosis, appropriate antimicrobial treatment should be commenced to prevent mortality and recurrence.

Key Words: *Burkholderia pseudomallei*, Epidemiology, Risk factors, Laboratory diagnosis, Treatment.

INTRODUCTION

As a member of the proteobacteria phylum, the genus *Burkholderia* comprises of 85 species⁽¹⁾. These species occupy surprisingly varied range of ecological niches – soil, water (including marine water), rhizosphere, animals and humans. Most of the species interact with plants and are phytopathogens. Among these, very few are pathogenic to humans and animals. Traditionally, *Burkholderia pseudomallei*, the etiological agent of melioidosis and *B. mallei*, the causative agent of glanders are two main species causing human infections.

Dr. Alfred Whitmore, a pathologist and his assistant C. S. Krishnaswami first described melioidosis as a "glanders-like" disease among morphin addicts in Rangoon, Burma, in 1911⁽²⁾. The disease was named melioidosis [in Greek "melis" (distemper of asses) and "eidos" (resemblance)] by Stanton and Fletcher in 1932. It is an aerobic, gram negative, motile bacillus exhibiting bipolar staining, often described as having "safety pin" appearance. When grown on solid media it has colony morphology ranging from

smooth to wrinkled form and cream to orange colour⁽³⁾. It is oxidase positive and assisimilitaes arabinose and motile.

EPIDEMIOLOGY

Melioidosis is regarded as endemic to Southeast Asia and northern Australia, corresponding approximately to the tropical latitudes between 20°N and 20°S. In north east Thailand, it is the most common cause of community acquired bacteremia and third most common cause of death from infectious disease after HIV/AIDS and Tuberculosis⁽⁴⁾. Sporadic melioidosis cases have been isolated in India⁽⁵⁾, Taiwan⁽⁶⁾, America⁽⁷⁾, China⁽⁸⁾, Cambodia⁽⁹⁾, Laos⁽¹⁰⁾, Malaysia⁽¹¹⁾. Cases are thought to be grossly underreported due to lack of awareness and incidence rates may be affected by imported cases travelling from endemic countries⁽¹²⁾.

INDIAN SCENARIO

In India cases have been reported from Tripura in the north to Kerala in the south. The first case was reported in India in 1991, in Dapoli Taluk of Maharashtra⁽¹³⁾. It no longer remains an exotic disease as numbers of cases reported are increasing in

number especially from west coast (5, 14 to 18) and central India.^(13,20) Though majority of the cases are seen mainly in adults, even children and neonates are also affected^(15, 16,21). Despite disputes regarding the etiology of outbreaks of plague in Maharashtra and Gujarat in 1994, it is likely the etiology was *B. Pseudomallei*⁽²²⁾.

ENVIRONMENTAL MICROBIOLOGY

B. pseudomallei can be isolated from soil and water and mode of infection is by inhalation, ingestion or inoculation of wounds⁽²³⁾. It is a resilient organism, capable of surviving extreme conditions, including prolonged nutrient deficiency of durations of up to 10 years, antiseptic and detergent solutions, acidic environments (pH 4.5 for up to 70 days), dehydration (soil water content of <10% for up to 70 days) but not exposure to UV light⁽²⁴⁾. It is possible that harsh environmental conditions confer a selective advantage for the growth of *B. pseudomallei*⁽²³⁾.

Other environmental factors that may influence the distribution of *B. pseudomallei* may include physical factors such as humidity, UV radiation, and temperature; chemical factors such as soil composition, other vegetation, and the use of fertilizers; and recent soil disturbances such as excavation and ploughing⁽²⁵⁾. The implications of global climate change on the epidemiology of melioidosis are still a mystery. Most of the cases are reported during monsoons and common in rice paddy workers.

HUMAN RISK FACTOR

A number of risk factors for developing melioidosis have been defined in several studies. Patients with diabetes mellitus, in particular, have a high incidence of melioidosis, with up to 60% of patients having pre-existing or newly diagnosed type 2 diabetes⁽²⁶⁾. Although it was suggested that insulin may have a direct effect on *B. pseudomallei*, the high incidence of type 2 diabetes, rather than type 1 diabetes, refutes this hypothesis as the mechanism of action. Several Studies have examined different risk factors in patients with melioidosis. In a Thai study, diabetes, thalassemia, chronic lung and

renal disease, alcoholism and occupational exposure to surface water were all associated with an increased risk of melioidosis⁽²⁷⁾.

CLINICAL PRESENTATION

Melioidosis is primarily seen to affect individuals who are in contact with soil and water. Since up to 80% of patients with melioidosis have one or more risk factors, it has been suggested that melioidosis should be considered more as an opportunistic organism that is unlikely to produce fatal outcome in a previously healthy person, provided that diagnosis and appropriate treatment is been given early. In a study on incubation period for melioidosis was been evaluated, in which 25% of patient who could recall a specific event such as injury had clinical manifestation 1 to 21 days (mean, 9 days)⁽²⁸⁾. Duration of incubation period depends on the infective dose, strain virulence, mode of infection and the risk factors in the individuals. Infection with high inoculum may result in a very short incubation period of less than 1 day as document in a near drowning event⁽²⁷⁾ whereas the longest incubation period been recorded was apparently 62 years⁽²⁸⁾.

Manifestation produced by melioidosis is varied and may mimic various other infections such as tuberculosis, dengue, leptospirosis, enteric fever etc. *B. pseudomallei* infection has protean clinical manifestations and degree of severity may vary from an acute fulminant septicemia to a chronic infection. They may present with pneumonia, genitourinary infections, skin infection, bacteremia without evident focus, septic arthritis or osteomyelitis and neurologic involvement⁽²³⁾. Internal organ abscess and secondary foci in lungs, joints or bones are common. In Indian subcontinent, melioidotic lymphadenopathy was the third most common presentation after pneumonia and septic arthritis⁽⁵⁾. Recurrent melioidosis may occur in approximately 1 in 16 patients, often in the first year after initial presentation. Roughly a quarter of recurrences are due to reinfection, with remainder due to relapse from a persistent focus of infection⁽²⁹⁾.

Paediatric melioidosis is predominantly, a disease of apparently immunocompetent in contrast to the disease of adulthood where 80% of those affected have at least one risk factor like diabetes mellitus, chronic lung and renal disease, alcoholic disease affecting the host immunity. The paediatric melioidosis usually manifest as localised infection with or without predisposing factors⁽³⁰⁾. There is evidence of vertical transmission and transmission through breast milk.

VIRULENCE FACTORS

Several putative virulence factors have been characterised in *B.pseudomallei*. Roles for capsule, type IV O-PS, quorum sensing, Type Three Secretion System, flagella and pili in pathogenesis have been demonstrated. Other virulence factors have been characterised but with limited experimental evidence, such as LPS and secreted enzymes. Capsular polysaccharide (CPS) appears to have a role in environmental protection, immune system evasion and attachment to epithelial cells⁽³¹⁾.

LABORATORY DIAGNOSIS

Isolation of *B. pseudomallei* from blood, sputum or other sterile fluid using culture methods is the 'gold standard' of detection. *B. pseudomallei* are a small gram-negative rod with bipolar staining.

GROWTH CHARACTERISTICS

B. pseudomallei on Sheep blood agar and MacConkey's agar produces metallic sheen with dry wrinkled colonies. On Ashdown agar, purple, flat, dry and wrinkled cauliflower like growth colony is seen. Ashdown's Selective agar utilises the gentamicin and colistin resistance of *B. pseudomallei* and neutral red allows it to be distinguished from other bacteria⁽³²⁾.

Burkholderia pseudomallei are oxidase Positive, Motile, oxidative utilization of glucose, lactose and maltose, dihydrolyses arginine, reduces nitrate to nitrites with gas, liquefies gelatine, growth

at 42^o C and resistance to polymyxin B (300 µg/disc).The antibiogram may be helpful in distinguishing *B. pseudomallei*, which is usually resistant to aminoglycosides and colistin or polymyxin but susceptible to Amoxiclav, a pattern which is very unusual in other organisms.

Serological evidence of *B. pseudomallei* infection can be obtained by detecting antigens or antibodies raised against the organism in clinical samples. One antigen detection test uses latex beads coated with monoclonal antibody which recognises a 200 kDa surface antigen on *B. Pseudomallei*⁽³³⁾. Immunofluorescence can be used to detect *B. pseudomallei* in sputum, urine and pus, using whole-cell specific antibodies conjugated to fluorescein isothiocyanate (FITC)⁽³⁴⁾. Enzyme linked immunosorbent assays (ELISAs) have been developed to detect antibodies raised against *B. pseudomallei*, using antigens such as LPS, OmpA and BipB⁽³⁵⁾. Molecular methods, such as PCR-based diagnostic tests, overcome some of the limitations of serological tests. Primers targeting 16S rRNA demonstrated sensitivity of 100% on culture confirmed cases but low specificity, with positive results in 33% of patients without clinical melioidosis⁽³⁶⁾.

TREATMENT

Following diagnosis of melioidosis, appropriate antimicrobial treatment should be commenced. Resistance of *B. pseudomallei* to several antibiotics can make treatment problematic. Clinical trials have established that an initial parenteral IV treatment followed by an oral eradication treatment is the most efficacious regime.

B. pseudomallei is intrinsically resistant to many antibiotics, including third generation cephalosporins, aminoglycosides and penicillins. Due to high intrinsic resistance and improper identification of nonfermenting gram-negative bacilli, these can prove very difficult to treat and can result in death.

Table 1. Melioidosis treatment and dosage schedule

Antimicrobial	Dose
<i>Initial intensive therapy</i>	
Ceftazidime	50 mg/kg body weight (upto 2 g), every 6 – 8 hr
Meropenem	25 mg/kg body weight (upto 1 kg) , every 8 hr
Imipenem	25 mg/kg body weight (upto 1 kg) , every 8 hr
<i>Oral eradication therapy</i>	
Trimethoprim – Sulphamethoxazole Body weight	
> 60 kg	2 × 160 mg of TMP-800 mg of SMX (960 mg) every 12 hr
40 – 60 kg	3 × 80 mg of TMP-400 mg of SMX (480 mg) every 12 hr
< 40 kg adult	1 × 160 mg of TMP-800 mg of SMX (960 mg) or 2 × 80 mg of TMP-400 mg of SFX (480 mg) every 12 hr
< 40 kg, child	8 mg of TMP/kg-40 mg of SMX/kg, every 12 hr

Dose information from Peacock *et al* ⁽³⁷⁾ and Chetchotisakd *et al* ⁽³⁸⁾

If exposure to *B. pseudomallei* is known to have occurred, post-exposure prophylaxis is recommended ⁽³⁷⁾. This has been evaluated and treatment with TMP-

SMX 0, 10 and 24 h post infection resulted in 100% survival rate ⁽³⁸⁾. Amoxicillin - clavulanate is the treatment of choice for pregnant women and results in a similar mortality rate as treatment with ceftazidime.

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