REVIEW ARTICLE

Melioidosis

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ABSTRACT

Melioidosis is a disease of public health importance in South East Asia and northern Australia, caused by a Gram negative soil-dwelling bacterium, *Burkholderia pseudomallei*. Sporadic cases are reported from other parts of the world, including India, while detailed information is unavailable. The disease is mainly seen in the population who are in prolonged contact with soil and are diabetic. There is a close association of the disease with rainfall. The primary manifestation is pneumonia with or without septicaemia, but other organs like bone and joints, liver, spleen, lymph nodes may also be affected. Despite improvements in antibiotic therapy, melioidosis is still associated with a significant mortality attributable to severe sepsis and its complications. The accurate and practical diagnostic set up is still unavailable in many parts of India, which leads to under- or mis-diagnosis, and fatality. The disease needs more attention from the microbiologists, clinicians and researchers from India, since studies related to the pathogenesis and virulence factors, preventive measures, vaccine prospects, and better management may reduce the burden of the disease.

Key words: *Burkholderia pseudomallei*, India, Melioidosis

INTRODUCTION

In 1911, Indian bacteriologist C. S. Krishnaswami, under the guidance of the pathologist Alfred Whitmore, described a "glanders-like" disease among the morphia addicts in Rangoon, Burma.[1,2] It was the first description of melioidosis. They identified that the bacterium was not similar to that which causes glanders, since its growth was relatively rapid, was motile, and it did not produce Strauss reaction when injected into animals. More than 90 years before full genome sequencing of the bacteria, Whitomore and Krishnaswami appreciated correctly that they had discovered a new infection that was very close to glanders. Whitmore also showed that Koch's postulate could be fulfilled and proposed the name *Bacillus pseudomallei* for the newly discovered bacterium. By 1917, Krishnaswami had reported over 100 cases from Rangoon (5% of all post-mortem examinations).[3] Melioidosis was named from the Greek words "melis," which means "distemper of asses," and "eidoes," which means "resemblance," by Stanton and Fletcher in 1932.[4]

During the last century, the Gram-negative soil dwelling environmental bacterium had been named differently —*Bacillus whitmori*, *Bacillus pseudomallei*, *Malleomyces pseudomallei*, *Pseudomonas pseudomallei*, and since 1992, it has been called *Burkholderia pseudomallei*.[5]

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EPIDEMIOLOGY

In the latter half of the 20th century, melioidosis emerged as an infectious disease of major public health importance in South East Asia and northern Australia, corresponding approximately to the tropical latitudes between 20°N and 20°S. In Thailand, which has the highest number of melioidosis cases at present in the world, *B. pseudomallei* is widely distributed in soil, and in pooled surface water such as in rice paddies. Melioidosis was also recognized as a disease of veterinary importance in northern Australia, affecting animals and, less commonly, birds.[6] Other than *B. pseudomallei*, there is another species, *Burkholderia thailandensis*, which is closely related to *B. pseudomallei*.[7] It is less virulent and not found in Australia.

Other than Thailand, melioidosis cases have also been reported from PDR Laos, Vietnam, Malaysia, Singapore, Indonesia, Cambodia, PR China, Hong Kong, Taiwan, Sri Lanka, and southern India [Figure 1]. The global map on melioidosis continues to expand to Brazil, Pacific region of New Caledonia, Western

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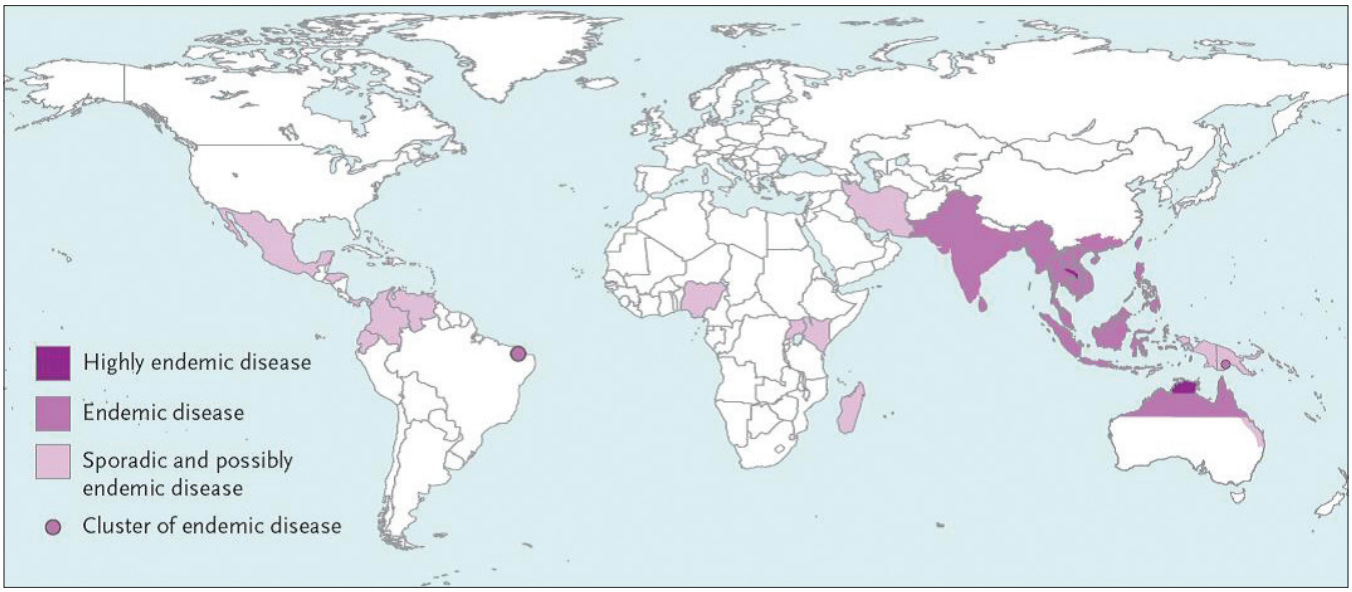

Figure 1: Global distribution of melioidosis[26]

Province of Papua New Guinea, especially after severe weather events like typhoons.[7] Sporadic cases have been reported from African countries like Nigeria, Gambia, Kenya, and Uganda, and from Latin American countries like Ecuador, Guadeloupe, and Aruba.[8-10] *B. pseudomallei* is considered to have potential in biological warfare, and is regarded as a potential bioterrorist weapon. It appears on the category B list of the critical agents published by the US Centers for Disease Control and Prevention.[6] There is a strong association between monsoon rain, occupational and recreational exposure to surface water and mud, and melioidosis, particularly in flooding of rice paddies and planting at the commencement of the monsoon season.[7] Other than rainfall, physical factors like humidity, UV radiation, and temperature and chemical factors like soil composition, other vegetation, and use of fertilizers may influence the distribution of *B. pseudomallei* in the soil.

Melioidosis is acquired from inhalation of contaminated dust or droplets, direct contact with contaminated soil or water through penetrating wounds and existing skin abrasions, aspiration of contaminated water, or ingestion.[7] In a few cases, it is reported to be nosocomial, sexually transmitted, or laboratory acquired.

INDIAN PERSPECTIVE

The Indian scenario of melioidosis is not very promising at present in regard to clinical and laboratory diagnostics. The first report of the presence of *B. pseudomallei* in India was from Scotland in 1953, followed by Germany in 1988. In both the cases, the travelers got infected in India but were diagnosed in their home countries.[11,12] The first report of melioidosis from India was by Raghavan *et al.* from Mumbai in 1991.[13] In 1994, the outbreak of so-called bubonic plague in Beed (Maharashtra) and pneumonic plague in Surat (Gujarat) also provoked the thought of an alternative diagnostic possibility of melioidosis.[14] It stimulated the detection of the widespread presence of melioidosis in various parts of India.

There are cases of melioidosis from different parts of India, especially from eastern and western coastal regions. There is, however, poor ascertainment of cases in India. Awareness of melioidosis among Indian clinicians and specialists in tropical medicine is very poor; in addition, the lack of proper laboratory facilities and expert clinical microbiologists contributes to under- or mis-diagnosis of the disease. Moreover, overgrowth of commensals from non-sterile sites (sputum), suboptimal selection of culture media, not using selective enrichment broth or agar, dismissal of the growth as contaminating non-fermenters from non-sterile sites, and discarding the culture plates by 3 days when the growth of *B. pseudomallei* may take 4-5 days in many occasions may lead to failure in growing *B. pseudomallei* in the laboratory. In suspected cases of melioidosis, the culture plates should be incubated for 7 days.

Clinicians, both at the hospitals and in the private setups, are not aware of the disease and are not following the recommended treatment protocol. Most

of the cases are wrongly diagnosed as tuberculosis, scrub typhus, leptospirosis, brucellosis, etc., which results in misdiagnosis or relapse. Unfortunately, the patient who does not receive proper treatment succumbs to septicemic melioidosis even when he avails himself of tertiary care facility. In the medical wards, especially in the medical Intensive Care Units (ICUs), the usual practice of the physicians is to initiate treatment with beta-lactam — beta-lactamase inhibitors (BL-BLIs). These combinations are not recommended for the treatment of melioidosis, but may, to some extent, decrease the bacterial load. However, the patient may come back with relapse. Even after the microbiological confirmation and recommendation of the proper treatment, it is found in some occasions that the treating physician refused to follow the recommendation, as the patient showed clinical improvement with other antibiotics. A similar scenario is encountered with surgical speciality, where the most common antibiotic used after any drainage of any superficial abscess is Amoxicillin–Clavulanic acid. Amoxicillin–Clavulanic acid is one of those antibiotics which showed high resistance with melioidosis. Most often, the abscess is drained and the patient is discharged with oral Amoxicillin–Clavulanic acid for 7-10 days. There is a high possibility that these patients may have a relapse in future. At present, there are few medical colleges and centers in Kerala, Karnataka, Tamil Nadu, Maharashtra, and Andhra Pradesh, which are diagnosing the disease and publishing case reports and series for awareness of the clinicians and microbiologists. Sero surveillance to study the exposure rate in the community and soil surveillance to study the bacterial load in the soil is still an unknown entity to Indian microbiologists and researchers.

PATHOGENESIS

B. pseudomallei, like many other soil bacteria, is a difficult organism to kill. It can survive in a variety of hostile conditions like nutritional deficiency, acid and alkaline pH, disinfectants, antiseptic solutions, exposure to many antibiotics, and extremes of temperature.[7] It is resistant to complement, lysosomal defensins, and cationic peptides, and produces proteases, lipase, lecithinase, catalase, peroxidase, superoxide dismutase, hemolysins, a cytotoxic exolipid, and at least one siderophore.[15-18] *B. pseudomallei* survives inside several eukaryotic cell lines and is seen within phagocytic cells in pathological specimens.[18-20] After internalization, it escapes from endocytic vacuoles and spreads from cell to cell for intracellular survival. The high mortality of *B. pseudomallei* infections is related to an increased propensity to develop high bacteremias (>1 cfu/ml).[21] The cell wall lipopolysaccharide (LPS) and the highly hydrated glycocalyx polysaccharide capsule of *B. pseudomallei* are important determinants of virulence, which help to form slime, protect the organism from antibiotic penetration, and alter the phenotypic character of the organism, resulting in reduced susceptibility to antibiotics (small colony variants).[22-25] Three Type III secretion systems (TTSS) help the intracellular survival and prevent the escape of the bacteria from endocytic vacuoles.[26] In addition to that, there are six Type VI systems, which also play a role in bacterial virulence. Capsular polysaccharide and lipopolysaccharide (O-PS I and O-PS II) and two other surface O-polyscaccharides (type III O-PS and IV O-PS), flagellin protein, and other cell wall proteins also contribute to the virulence.[6,26]

Septicemic melioidosis is associated with very high concentrations of both pro-inflammatory and anti-inflammatory cytokines — tumor necrosis factor (TNF), interleukin-6, interleukin-10, interferon-gamma, and interleukin-18.[27-30] The CXC chemokines, interferon-gamma — inducible protein 10 and monokine induced by interferon-gamma, and the serine protease granzymes A and B are also notably raised in septicemic melioidosis.[31,32] The concentrations of interleukin-6 or interleukin-10 are independent predictors of mortality.[30] Antibodies against LPS components have been demonstrated to be protective. Antibody level was the highest for IgG (IgG1 and IgG2).[33] Antibodies can persist variably over 3 years.

RISK FACTORS AND CLINICAL PRESENTATION

A number of risk factors for developing melioidosis have been described in various articles. Patients with diabetes mellitus, either preexisting or newly diagnosed type 2 diabetes mellitus, have a high incidence of melioidosis. Other than that, risk factors like thalassemia, renal disease, chronic lung disease, chronic alcoholism, occupational exposure to soil and surface water, elderly (>45 years) males, and immunosuppressed state due to corticosteroid therapy may make the population susceptible.[7] Incubation period is usually 1-21 days, but can be as long as months and even years.[26]

Clinical presentation of melioidosis varies widely. It varies from acute fulminant septic illness to a chronic infection (symptoms persisting more than 2 months and it accounts for 11% of all the cases).[26] The Vietnam War experience drew attention toward more chronic forms

of the infection or reactivations long after exposure. Such forms were described as "Vietnam time-bomb," which manifested later in life as a tuberculosis-like disease.[6] There may be a possibility that the acute form of septicemia missed diagnosis in the battlefield. The most common presentation is community-acquired pneumonia. However, many patients presenting with prolonged fever, weight loss, and suggestive chest X-ray findings are misdiagnosed as tuberculosis and may get antitubercular therapy. Other than that, there are patients with septic arthritis, multiple abscesses in liver, spleen, prostatic abscess (more in Australia), suppurative parotitis (more in Thailand), osteomyelitis, pyomyositis, cellulitis, fasciitis, skin abscesses or ulcers, and bacteremia with or without focus. On radiograph, Swiss-cheese appearance of the deep-seated abscess in liver, spleen, or in other organs is seen. Mortality rate in Indian setup varies from 7 to 20%.[34,35]

LABORATORY DIAGNOSIS

The protean manifestations of melioidosis often lead to clinical under-diagnosis of this fatal disease. Confirmation of melioidosis relies heavily on clinical microbiology laboratories and specifically on bacterial isolation by culture methods. Serological and molecular methods have been evaluated in clinical settings. However, culture is still considered the gold standard.

Culture of *B. pseudomallei*

Although *B. pseudomallei* grows on most routine laboratory media using standard techniques, isolation rates reflect the availability of laboratory facility and laboratory experience with this pathogen. Common specimens that yield *B. pseudomallei* on culture are blood, bone marrow, pus, fluid aspirates, tissue, throat swab, sputum, broncho-alveolar lavage (BAL) fluid, urine, ascitic fluid, etc. Standard specimen collection and transport principles are sufficient for recovery of this organism in clinical practice.

Blood and bone marrow cultures can be done using conventional or automated blood culture system. Growth occurs within 5 days in conventional cultures and in 2 days in automated cultures. Hence, extended incubation is not required.[36]

Isolation and identification of *B. pseudomallei* from non-sterile sites is difficult due to lower number of pathogen and overgrowth by commensal flora.[37] Presence of closely related *Burkholderia* spp. in specimen from non-sterile sites and atypical colony morphology of some *B. pseudomallei* strains compound the practical difficulties of diagnostic laboratories. This problem can be overcome by the use of selective pre-enrichment broth or selective agar (Ashdown's agar) in addition to routine media like sheep blood agar and McConkey agar.[38]

All plates are incubated at 35°-37°C for up to 5-7 days. Any culture suspected of *B. pseudomallei* should be handled in class 2 biological safety cabinets (BSC II). Colonies are often small, smooth, and creamy in the first 1-2 days on sheep blood agar, which gradually change after a few days to dry and wrinkled colonies. Distinctive musty or earthy odor is encountered while opening the Petri plates. Pink or colorless colonies are seen on MacConkey agar after 1-2 days with metallic sheen. After 3-4 days, the colonies may become dry and wrinkled. Ashdown medium contains tryptase soy agar with glycerol, crystal violet, neutral red, and Gentamicin (4 mg/l). Colonies of *B. pseudomallei* on Ashdown agar are pinpoint, clear, and pale pink at 24 h, changing to pinkish purple, flat, and slightly dry with sheen in the next 2 days. After 5-7 days, the colonies get characteristic wrinkled appearance (rugose, or cornflower head) and they take up crystal violet from the medium [Figure 2]. A modified Ashdown medium with colistin is now commonly used.[39]

Identification of *B. pseudomallei*

Few simple screening tests are employed for presumptive identification of *B. pseudomallei* in endemic areas. Colonies with metallic sheen, short Gram-negative bipolar stained bacilli [Figure 3], positive oxidase test, and resistance to colistin and Gentamicin are further characterized for definitive identification of *B. pseudomallei*. The common tests like oxidative utilization of glucose in OF test medium, nitrate reduction, negative indole test, alkaline slant/no change in butt in triple sugar iron (TSI) media, negative H_2S production, positive arginine dihydrolase, negative lysine and ornithine decarboxylase, and negative OF-arabinose help to strengthen the identification.[40,41]

Commercial identification systems like API20NE and Vitek 2 may be used; however, the conflicting results in identification,[42,43] limited availability, and cost have marginalized their usefulness in India. Latex agglutination test with monoclonal antibodies against *B. pseudomallei* is a reliable and rapid method to identify *B. pseudomallei* when available. This test reliably differentiates *B. thailandensis*, a non-pathogenic species often mistaken for *B. pseudomallei*.[44,45]

Antibiotic sensitivity test

In Kirby Bauer disk diffusion testing on Mueller

Figure 2: *Burkholderia pseudomallei* colonies on Ashdown agar

Hinton agar, *B. pseudomallei* shows susceptibility to Ceftazidime, Amoxicillin–Clavulanic acid, Piperacillin, Tetracycline/Doxycycline, Trimethoprim — Sulfamethoxazole, Imipenem/Meropenem; variable susceptibility to Ciprofloxacin; and resistance to Amikacin, Gentamicin, Netilmicin, Tobramycin, Colistin, and Polymyxin B.[40]

Rapid detection methods

Although a variety of rapid antigen detection methods have been studied, none are commercially available yet. Antigen tests have been developed for use directly on specimens or in blood culture supernatant. Among these, latex agglutination is evaluated for identification of culture or blood culture supernatants in Thailand and demonstrated a sensitivity 95.1-100% and a specificity of 99.7-100%.[46,47] Similar results are shown in a study from Vellore using in-house co-agglutination test and latex agglutination test.[48]

In Thailand, the results of rapid immunofluorescence (IF) test and those of an existing IF method were prospectively compared with the culture of various clinical specimens from patients with suspected melioidosis. The sensitivities of both IF tests were 66%, and the specificities were 99.5 and 99.4%, respectively.[49,50]

Antibody detection

Indirect hemagglutination assay (IHA) remains the most widely used test despite its poor sensitivity and specificity. The use of IHA is problematic in areas of endemicity due to background seropositivity as a result of environmental exposure to *B. pseudomallei*. These facts have limited the diagnostic utility of IHA in acute melioidosis. Enzyme-linked immunosorbent assays (ELISAs) used on affinity-purified antigen, crude antigen, LPS, and extracellular polysaccharide (EPS) have been validated in clinical context with varied ranges of sensitivity (63.9-82.4%) and specificity (71.1-82.1%).[51]

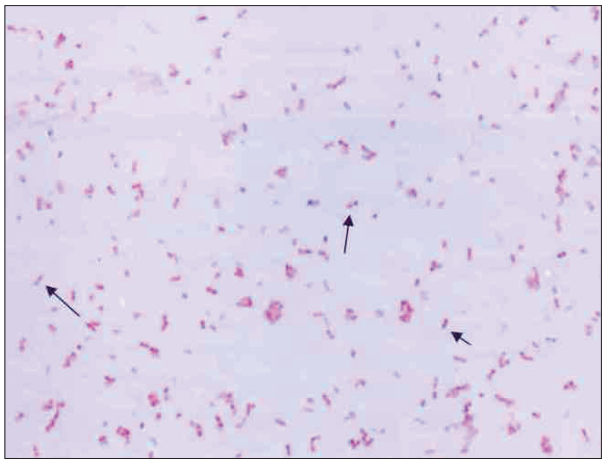

Figure 3: Bipolar stained' *Burkholdria pseudomallei* under Gram stain

Immunochromatographic tests have also demonstrated similar results and presented an enhanced performance over IHA.[52] Overall, IgG detection and not IgM appears to be a more sensitive indicator of clinical disease.[53]

Molecular diagnosis

Delay in obtaining the culture results and inability of the serological methods in achieving accurate diagnosis have led to the search for accurate and rapid tools such as polymerase chain reaction (PCR). Primers targeting 23S rRNA, 16S rRNA, and 16S and 23S RNA junction and *TTS1* gene have been evaluated both on bacterial cultures and clinical specimen. All PCR protocols showed accurate results in confirming the bacterial isolate after culture, while their sensitivity and specificities varied on testing the clinical specimen directly. Low number of bacteria in blood specimen, presence of PCR inhibitors in blood, and probable latency of the bacterium within macrophages without an active disease are the possible explanations. Overall, molecular detection is still not superior to culture in case of *B. pseudomallei*.[54-56]

ANTIBIOTIC TREATMENT

Melioidosis may have a protracted course and a chance of relapse if proper antibiotic treatment is not continued for an adequate period of time. The bacteria are inherently resistant to many antibiotics that are commonly used against Gram-negative non-fermenters — like Penicillin, Ampicillin, first generation and second generation cephalosporins, Gentamicin, Tobramycin, Streptomycin, and Polymyxin. Ertapenem, Tigecycline, and Moxifloxacin — like Tetracyclines, Chloramphenicol, the quinolones, and

Table 1: Antimicrobial treatment of melioidosis[26]

Antimicrobial drug	Dose
Initial intensive therapy*	
Ceftazidime	50 mg/kg of body weight (up to 2 g), every 6-8 h, or
Meropenem	25 mg/kg (up to 1 g), every 8 h, or
Imipenem	25 mg/kg (up to 1 g), every 6 h
Oral eradication therapy**	
TMP–SMX	
Body weight	
>60 kg	2 × 160 mg of TMP-800 mg of SMX (960 mg), every 12 h
40-60 kg	2 × 80 mg of TMP-400 mg of SMX (480 mg), every 12 h
<40 kg, adult	1 × 160 mg of TMP-800 mg of SMX (960 mg), every 12 h, or 2 × 80 mg of TMP–400 mg of SMX (480 mg), every 12 h
<40 kg, child	8 mg of TMP/kg-40 mg of SMX/kg, every 12 h

*Intensive therapy is defined as intravenous administration of one of the listed medications for a period of 10-14 days. Four or more weeks of parenteral therapy may be necessary in patients with severe disease (e.g. those with ongoing septic shock, deep-seated organ abscesses, extensive lung disease, septic arthritis, osteomyelitis, or neurologic melioidosis). The addition of TMP–SMX should be considered for patients with neurologic, prostatic, bone, or joint melioidosis. A switch to Meropenem is indicated if the clinical condition worsens with the administration of Ceftazidime (e.g. organ failure develops), if a new focus of infection develops during treatment, or if repeated blood culture remains positive at 7 days

**Oral therapy is typically required for 3-6 months. If the organism is resistant to TMP–SMX or the patient has unacceptable adverse events in response to medication, the second-line choices are Amoxicillin–Clavulanic acid and Doxycycline. Amoxicillin–Clavulanic acid is recommended at a dose of 20 mg of Amoxicillin and 5 mg of Clavulanic acid per kilogram of body weight given orally, three times daily

Ceftriaxone, do not appear to be clinically useful in the intensive phase.[7]

The treatment of melioidosis is divided into two phases: Intensive phase, consisting of in-patient treatment for at least 10-14 days with Ceftazidime or carbapenems (Imipenem or Meropenem) and Eradication phase, consisting of treatment with oral Trimethoprim–Sulfamethoxazole (TMP–SMX) for 3-6 months [Table 1].

PREVENTION

Melioidosis is a preventable disease, and there should be proper guidelines for the people who are prone to suffer from the disease in endemic areas. Even tourists traveling to endemic areas should be properly cautioned against barefoot walking and recreational activities in water. People with diabetes, renal transplant, on chronic steroids, cystic fibrosis, in regular contact with soil and surface water should be guided to take proper precautions, like wearing protective footwear, mask during rainy season while going to the paddy fields, or when there is a chance of heavy wind and rain. Cut injuries in the limbs should be protected from exposure to the soil. Laboratory technicians and research workers should be trained to use aprons, mask, and gloves while working with the sample and culture in the laboratory, to expose the culture inside BSC II facility, and should not try to sniff the colonies from the culture plates.

Vaccine

There is no human vaccine available so far. However, there are researches in animal models involving the use of live attenuated, subunit, plasmid-based DNA, and killed whole-cell vaccine.

FUTURE OF MELIOIDOSIS RESEARCH IN INDIA

The present research initiatives in India for melioidosis are very much local and only focused in publishing case reports and series. Clinicians and policy makers have no clue about the disease burden in the community. Most often, the diagnosis of the disease in the laboratory is accidental. There should be a regular communication among the microbiologists in India, who are interested in the research of the disease. There should be a forum where the clinical and laboratory aspects of the disease can be discussed, awareness program for the doctors and common people in the society for better public health can be formulated, and expert opinions from all around the world can be availed of. The Department of Microbiology, Kasturba Medical College, Manipal is taking initiatives to bring all the interested researchers under one umbrella. Moreover, it may play a role of a reference laboratory in future, where bacterial strains can be sent for confirmation after presumptive diagnosis is made in the respective institutions.

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