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MELIOIDOSIS: AN EXOTIC REEMERGING INFECTIOUS DISEASE

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ABSTRACT

Burkholderia pseudomallei (formerly*Pseudomonas pseudomallei*) is the causative organism and its role in the development of Melioidosis and septicemia is known to scientists for a century .Despite the introduction of many new antimicrobial agents with enhanced activity against *B .pseudomallei* ,the high mortality in *Septicemic melioidosis* ,over the years continues .The disease is endemic in South East Asia and Northern Australia. Thailand has reported highest number of cases. Melioidosis also been documented in many other countries In endemic areas organism is found in the surface water and soil and are usually transmitted to humans by cutaneous or by inhalation. Clinical presentation range from septicemia, skin ulcers ,or chronic pneumonia mimicking tuberculosis, with abscesses in multiple internal organs .Therapy of melioidosis remains an unresolved problem ,with high mortality rate in septicemic patients with defined risk factors such as diabetes ,alcohol abuse and renal disease. *B. pseudomallei* are generally susceptible to ceftazidime, Imipenam, Meropenam, and maintenance therapy with sulphamethoxzole-trimethoprim and doxcycline. *B.pseudomallei* is commonly resistant to ampicillin, first generation and second generation cephalosporins and, gentamicin, and tobramycin. Recurrent disease are common in all varieties of melioidosis because of failure of eradication or noncompliance .Melioidosis is a Global reemerging infectious disease .This paper reviews the pathogenesis ,clinical manifestation and the role of melioidosis as an reemerging infectious disease.

KEYWORDS: *Burkholderia pseudomallei*, Pathogenesis, Clinical Manifestation, Therapy

INTRODUCTION

Melioidosis is causedby a widely distributed Gram negative bacterium *Burkholderia pseudomallei* (formerly *Pseudomonas pseduomallei)* was first discovered in Burma (now Myanmar) by Whitmore and Krishnaswami in 1912[1].Stanton *et al.*, predicted that disease would prove to be more common than appreciated at that time [2].The disease is endemic in South East Asia and Northern Australia. Melioidosis cases have been reported in human and animals in Malaysia, Singapore, Vietnam, and Indonesia [3]. Thailand, has reported highest number of cases. Melioidois also been documented in China, Taiwan, Brunei, Philippines, cases have been reported from Sri Lanka, Bangladesh and Pakistan as well as in travelers and soldiers who have resided in endemic areas . Cases of melioidosis are increasingly documented outside the classic endemic region of Southeast Asia, Australasia, the Indian subcontinent and China. Cases of melioidosis also have been reported from Middle East, East Africa, the Caribbean and Central and South America [4]. The disease remain poorly understood although recognized for a century .The organism is found in the surface water and soil and are usually transmitted to humans by cutaneous or respiratory route. Clinical manifestations range from sub clinical infection to septicemia that resemble to disseminated or localized supportive infection due to various pathogens. Melioidosis has enormous clinical diversity, spanning asymptomatic infection, skin ulcers or abscesses, chronic pneumonia mimicking tuberculosis, and fulminant septic shock with abscesses in multiple internal organs[5].Most disease is from the recent infection, but with reactivation is described from 26 years to 62 years after exposure or after leaving the area [6, 7].

BURKHOLDERIA PSEUDOMALLEI

Burkholderia pseudomallei are a small gram-negative, oxidase – positive, motile, aerobic bacillus with occasional polar flagella.On staining, a bipolar "safety pin" pattern is seen. The organism is easily recovered on standard culture medium but may misidentify as *B.cepecia, P.stutzeri*, or other *Pseudomonas s*pecies. The organism is present in soil and surface water in endemic regions. Human and animals are infected by percutaneous inoculation, inhalation, or ingestion .Occasional laboratory-acquired infections are described[5].*B.pseudomallei* is a normal inhabitant of soil and water and indigenous to the natural environments of Southeast Asia and northern Australia. Though moist soil contains a greater number of organism, they can survive the dry seasons for many months [8].Such persistence has been demonstrated by field survey and in laboratory model .In the rainy season, the organisms in the soil may come up to the surface of water table and then multiply in the stagnant water and muddy sites such as rice paddies[8].One of the interesting aspects of *B.pseudomallei* in natural environments is that the pH varying with depth of soil has little or no effect on their growth[9].

In addition to endemic melioidosis, there are several documented situations where melioidosis became established in non-tropical locations. In France in 1970s, the cases of melioidosis occurred in animals in a Paris Zoo, with spread to other equestrian clubs. In addition to fatal animal and human cases there was extensive soil contamination persisting for years. *B.pseudomallei* was considered likely to have been introduced by importation of infected animals [3]. Yabucchi *et al*, in some ingenious experiments, have shown that various strains of *B.pseudomalleii* were still viable after 180 days without appreciable decrease of their count at 4-5 C .These facts would support the survival of the organism in areas outside the tropics [10].

Researchers in Australia have confirmed that in addition to widespread presence of *B.pseudomallei* across the tropical north, a number of temperate locations well south of tropics have been identified where melioidosis has occurred in humans and in animals. These include hobby farms in an area of southwest river valley around Ipswich (27.5) in southeast Queensland [11, 12] The recent observation that .*B.pseudomallei* can survive inside free living amoeba provides an alternative explanation and incidentally demonstrate a high degree of adaptation to an intracellular environment[13].

TRANSMISSION

Recent studies from Malaysia ,Thailand and Australia ,have found that organism is more common in cleared ,irrigated sites such as rice paddies and farms [14,15].It has been suggested that increase in melioidosis cases in Thailand may partly be the consequence of increased exposure to bacteria resulting from changes in behaviors, such as farming techniques[16].In Australia, *B. pseudomallei* has been found most commonly in clay soils to a depth of 25 to 45 cm, and it has been proposed that bacteria move to surface with rising of water table during the wet season[9]. An alternative explanation for variable bacterial presence found is that during times of stress, such as prolonged dry seasons, *B.pseudomallei*may persist in soil in a viable but in dormantstate [13]. Differential gene variation may allow such environmental bacteria to respond and adapt to different environmental conditions .The role of biofilms in the persistence of *B .pseudomallei* environment, as well as in human hosts, requires further study [17].There is increasing interest in the intracellular survival of *B.pseudomallei,* and it has been proposed that an ecologic niche for bacteria in the environment may be in environmental protozoa or fungi[13].

In most endemic region, there is a close association between melioidosis and rainfall. In Thailand and in Australia, 75% to 85 % of cases, respectively, have occurred in wet season [18]. Researchers in Sabah, Malaysia reported no seasonal variation in the occurrence of cases [19].Although early animal studies showed infection with B.pseudomallei through oral or nasal exposure and from ingestion, morerecent reviews have considered that most human cases are from

percutaneous inoculation of *B.pseudomallei* after exposure to muddy soil or surface water in endemic locations [20].Singapore the city state is highly urbanized with 88 % of the population living in flats .Unlike those rural communities ,there is less opportunity for the population to have direct contact with contaminated soil and surface water ,has reported annual incidence rate of 1.3 per 100,000 population ,with case mortality 66.7 % in septicemia and 32.9 % in without septicemia [21].In a study in Sabah ,Malaysia have reported 220 cases of melioidosis with a case mortality of 75.0% in septicemia, compared to 65 % in septicemia in West Malaysia [19].

Human to human transmission of melioidosis has been described through venereal transmission from a patient with chronic prostatitis due to *B.pseudomallei* to his wife, and in a diabetic woman who cared for her brother with septicemia melioidosis [22, 23]. Melioidosis after near drowning is well documented, with probable infecting event being aspiration [24]. Melioidosis by inhalation route is well documented in Vietnam for soldiers exposed to dust raised by helicopter rotor blades [25]. Melioidosis by inhalation has been reported, a British RAF helicopter pilot while serving in Sabah, Malaysia [26].

Incubation period for melioidosis is influenced by inoculating dose, mode of infection host risk factors, and probably differential virulence of infecting *B. pseudomallei* strains. Onset of melioidosis within 24 hours has been presumed aspiration after near drowning and, in some cases ,after severe weather events .In 25 cases of acute melioidosis in which a clear incubation period could be determined between the inoculating injury and the onset of symptoms ,the incubation period was 1 to 21 days (mean,9 days) ,which is consistent with series of nosocomial cases from Thailand ,in which incubation period was 3- 16 days (mean.9.5 days) [27,28].

PATHOGENESIS

Little is known concerning the pathogenic mechanism (s) involved in the successful infection by *B.pseudomallei*. The present knowledge on the pathogenicity of the causative bacteria is inadequate to explain all of the disease features presented during both acute systemic and pulmonary melioidosis [29].Acute melioidosis can manifest itself in the form of pulmonary and systemic infection [30]. In vivo studies by intratracheal introduction of the organism into hamster lungs fail to reflect the requirement for minimal infectious dose of the organism in natural infection[29] Serology studies have shown that most infection with *B.pseudomallei* is asymptomatic.Studies in Thailand confirm that most rural population is seropositive by indirect hemaggultination (IHA) with most seroconversion occurring between 6 months and 4 years of age Although the melioidosis occurs in all age groups, severe clinical disease such as septicemic pneumonia is seen only in those with risk factors such as diabetes, renal disease and alcoholism[19] Studies in Sabah, Malaysia reported 15.8 % seropositive in army recruits [31].

Infection by inhalation, bacterial load on exposure (inoculating dose) and virulence of the infecting strain of *B. pseudomallei* are also likely to influence the severity of the disease .However, it has been noted that despite the large bacterial load in severely ill patients with septicemic pulmonary melioidosis, person- to person transmission is extremely unusual. This together with the rarity of fulminant melioidosis in healthy people supports the primary importance of host risk factors for development of melioidosis. Furthermore although it is clear from laboratory studies of isolates of *B.pseudomallei* from animals, and humans, and the environment that virulence differs among *B .pseudomallei* isolates [32]. The importance of this variation in virulence in determining clinical aspects of melioidosis remains unclear. Molecular typing and whole genome sequencing and subsequent molecular studies have shown that *B.pseudomallei* has two chromosomes, multiple genomic islands that are variably present in different strains and have a great propensity for horizontal gene transfer [33].Further studies are required to unravel the global phylogeny and evolutionary history of

B.pseudomallei and related species and to determine which genes orgene cluster may be critical for pathogenesis and disease presentation and outcome[34].

There have been a number of studies showing elevated levels of various endogenous inflammatory mediators and cytokines to be associated with severity and outcomes of melioidosis .Nevertheless, whether these elevated cytokines are a cause or result of severe disease is not established. In Thailand, there was an association of severe melioidosis with tumor necrosis factor (TNF)- alpha gene allele 2, which is linked to higher constitutive and inducible production of TNF-alpha [35].However, in a mouse model of melioidosis ,neutralization of TNF-alpha or interleukin (IFL)-12 increased susceptibility to infection in vivo, and interferon –alpha (IFN- alpha) was found important for survival ,with mice treated with monoclonial anti-IFN-alpha dying more quickly [36]. A role for Toll-like receptors in innate immune response in melioidosis has been proposed [37].There are ,therefore ,important host protective mechanisms against *B .pseudomallei* in cytokines responses as well as potentially detrimental ones ,with the timing of cytokine release and the balance between pro-and anti-inflammatory responses likely to determine the severity of disease and outcome of infection[38,39].The extent to which host polymorphisms in immune response contribute in comparison to differences in organism virulence ,infecting dose of *B.pseudomallei* ,and defined host risk factors such as diabetes remains to be clarified .Nevertheless ,the predominant association with fatal melioidosis is the presence of defined patient risk factors [40].Although a vigorous cell-mediated immune response may protect against progression of disease there is no definitive evidence for the development of immunity from melioidosis after natural exposure to *B. pseudomallei*,and reinfection can occur with different strain of *B. pseudomallei* after successful treatment of melioidosis. Evidence suggests that there may be a predisposition to melioidosis may reflect diabetes, alcohol access, or chronic renal disease, which reflects impairment of their neutrophil and other phagocytic cell functions, such as mobilization, delivery, adherence, and ingestion. Melioidosis has also been described in chronic granulomatous disease [41-43].

Dormancy and Recrudescence of Melioidosis

There is a firm experimental foundation showing that *B. pseudomallei* may persist for long periods in vivo in a viable state .*B. pseudomallei* is a facultative intracellular pathogen that can invade and replicate inside various cells, including polymorphonuclear leukocytes and macrophages and some epithelial cell lines [44].

Animal models have been unable to confirm a clinically relevant exotoxin for *B. pseudomallei*[45].However resistance to human serum conferred by lipopolysaccharide(LPS), and the ability of *B.pseudomallei* to survive intracellularly (conferred by capsular polysaccharide) appear to be critical in the pathogenesis of melioidosi [46,47]..Type 111 secretion system in *B. pseudomallei* have also seems to be important in cell invasion and intracellular survival. Quorum sensing may play an important role in many aspects of virulence of *B. pseudomallei*, including cell invasion, cytotoxicity and antimicrobial resistance [48,49].

Intracellular survival of *B. pseudomallei* in human and animal hosts is likely to explain the ability for latency .After internalization, *B. pseudomallie* escapes from endocytic vacuoles into the cell cytoplasm, and induction of actin polymerization at one bacterial pole leads to membrane protrusion, with cell- to cell spread involving these actin tails [33,37].Additional survival factors are the ability of *B. pseudomallei* to form antibiotic resistant small colony variants and the ability of mucoid variants with large extracellular polysaccharide glycolic structures to form biofilim–encased micro colonies that are also relatively antibiotic resistant [44].The chronicity and recrudescence of the disease in the majority of *B. pseudomallei* infection must relate to the ability of the organism to persist in the face of an immune response and manifest into full-blown infection in immunocompromised hosts with underlying ailments such as diabetes , and lung

cancer [45].The long persistence of *B.Pseudomallei* cells in tissues can produce granulomas which are not different from those of tuberculosis on histological grounds alone [46].

CLINICAL PRESENTATION

The earliest description of melioidosis documented the fulminant end of the clinical spectrum, with abscesses throughout both lungs and in many organs[1]. Melioidosis has been classified as acute, subacute, the chronic [26]. In a Study in Thailand of 898 patients 16.8 % were children, and 83.2 % were adults It was common in 40- 60 age groups .Male to female ratio was 1.4:1 [47]. The infectious disease Association of Thailand has summarized 345 cases in four categories,a multifocal infection with septicemia (45 % of cases,87 % mortality. b) localized infection with septicemia (12 % cases, 17 % mortality). c) Localized infection (42 % cases, 9 % mortality) d) transient bacteremia (0.3 %) [48].More recent bacteremia and overall mortality rates have been, respectively,60 % and 44 % in Thailand,46 % and 19 % in Australia ,and 43 % and 39 % in Singapore [5,39], in Sabah Malaysia 75 % mortality with septicemia melioidosis[19]. Pneumonia is the commonest clinical presentation of patients with melioidosis in all studies, accounting for half of cases. Secondary pneumonia after another primary presentation occurs in around 10 % of cases.

Acute melioidosis pneumonia has spectrum from fulminant septic shock (mortality up to 90 %); to mild undifferentiated pneumonia, which can be acute or sub-acute in nature, with little mortality. Septicemia patients present acutely unwell with high fevers and prostration and often little initial cough or pleuritic pain. On chest radiographs, diffuse nodular infiltrates often develop throughout both lungs and they coalesce, cavitate, and progress rapidly, consistent with the caseous necrosis and multiple metastatic abscess formation seen at autopsy.

Nonsepticemic patients with pneumonia and some with septicemia pneumonia have a more predominant cough, with productive sputum and dyspnea, and their chest radiograph show discrete but progressive consolidation in one or more In endemic regions acute pneumonia with upper lobe consolidation warrants consideration of melioidosis, although lower lobe infiltrate also common [49].

Recent studies confirm that *B.pseudomallei* can colonize airways and cause disease in patients with cystic fibrosis (CF) and bronchiectasis .The infection is similar to infection with *B.cepacia* complex in CF with more rapid deterioration of lung function [50].Three differences have been noted in clinical presentation between Thailand and tropical Australia:

First,supportive parotitis accounts for up to 40% of melioidosis in children in Thailand, but very rare in Australia [51,52].

Second, prostatic melioidosis is well recognized but uncommon, except in Australia, where routine abdominopelvic CT scanning of all melioidosis cases has shown prostatic abscess to be present in 18 % of all male patients with melioidosis[40].

Third, neurologic melioidosis accounts for around 4 % of cases in northern Australia, with the distinctive clinical features being brain stem encephalitis often with cranial nerve palsies (especially the seventh nerve), together with peripheral motor weakness, or occasionally just flaccid paraparesis alone. CT scan is often normal but dramatic changes seen on magnetic resonance imaging [53].

It has long been recognized that .*B pseudomallei*, like tuberculosis, has the potential for reactivation from the latent focus, usually in the lung – hence the concern of the "Vietnam time bomb" in returned soldiers .Latent period from exposure to *B. pseudomallei* in an endemic region to onset in a non-endemic region have been documented as being as long as 62 years [7].

LABORATORY DIAGNOSIS

A definitive diagnosis of melioidosis can only be made with the isolation and identification of *B.pseudumallei*.The organism can be isolated on routine laboratory media,but Ashdown's selective media a gentamicin containing media isuseful in specimens with mixed normal organisms [54].*B.pseudomallei* can be identified by combining the commercial API 20 NE biochemical kit with a simple screening system involving the Gram stain, oxidasereaction, typical growth characteristics,and resistance to certain antibiotics[55].

THERAPY FOR MELIOIDOSIS

Melioidosis is recognized as an important infectious disease in South East Asia and in northern Australia. Antimicrobial therapy of melioidosis presents a problem because of resistance of the organisms to the more conventional antibiotic, the extreme variation in the severity and course of infection in different patient, the known tendency to relapse and reported emergence of resistant strains.

Intensive Therapy 10-14 Days

Ceftazidime (50mg/kg, up to 2 g) every 6 hrs.

Or Meropenam (25 mg/kg, up to I g) every 8 hrs.Or Imipenem (25gm /kg, up to I g) every 6 hrs.

Any one of the three may be combined with

Sulphamethoxzole-trimethoprim (40/8 mg/kg up to 1600/320 mg) every 12 hr

(Recommended for neurologic cutaneous, bone and prostatic melioidosis)

Maintenance Therapy (Minimum 3 Months)

Sulphamethoxzole-trimethoprim (40/8mg /kg up to 1600/320 mg) every 12 hr

With or without Doxycycline (2.5mg/kg up to 100 mg) every 12 hr[5].

CONCLUSIONS

Melioidosis is endemic in Southeast Asia and northern Australia. Thailand has reported highest number of cases.The manifestation of melioidosis in human varies from sub-clinical to protean, overwhelmingly resembling other acute bacterial infections. The spectrum of disease in Malaysia is similar to that seen in Thailand and Australia. Most of the strains are susceptible to 3rd generation cephalosporinsand tetracycline .Ceftazidime is the drug of choice with tetracycline or Co-trimoxazole added. As the septicemia melioidosis has high mortality, high clinical suspicion is required and appropriate antibiotic therapy should be instituted.

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