IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 5, Issue 2 (February 2015), PP. 13-19

Clinical manifestations, diagnosis, and treatment of Melioidosis

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ABSTRACT: *Meliodosis is endemic in Southeast Asia,northern Australia, and Brazil. Northeast Thailand has the highest incidence of melioidosis. Septicemic meliodosis has high mortality,87% in Thailand,75%in East Malaysia,39% in Singapore and 19% in Australia. Localised melioidosis has lower mortality. Burkholderia pseudomallei is found in soil and water,a history to contact with soil or surface water almost invariable with melioidosis. Diabetes mellitus, renal failure,thalassemia,haematological conditions, malignancy,steroids therapy,alcoholism and penetrating injury are the main risk factors.Clinical spectrum of melioidosis,being the fulminant end of clinical manifestation, with abscesses throughout both lungs and in many organs.Ashdown's medium containing gentamicin, is a selective medium for growth of B.pseudomalleui. Antibiotics of choice include, ceftazidime, imipenem for initial intensive intravenous therapy for 10 to 14 days,sulfamethoxazole-trimethoprim and doxycycline for eradication therapy for minimum 3 months.Septicemic melioidosis has high mortality, high clinical suspicion is required, and appropriate empirical antibiotic therapy should be instituted for a better outcome.*

KEYWORDS: *Melioidosis, Burkholderia pseudomallei, Diagnosis, and Treatment*

I. INTRODUCTION

Melioidosis is endemic in parts of southeast Asia, including,Thailand, northern Australia, and is an emerging infectious disease in Brazil, sporadic cases has been reported from other parts of the world [1,2,3]].Northeast Thailand has the highest incidence of melioidosis in the world(21.3 cases of melioidosis per 100,000 people per year)[4].Melioidosis is a recognized disease in animals,including cats,goats, sheep. horses[5]. *Burkholderia pseudomallei* is normally found in soil and water, a history to contact with soil or surface water is, therefore, almost invariable in patients with melioidosis [6].Also implicated are severe weather events such as flooding, tsunamis, and typhoons[7,8,9].The disease is associated with rainy season, with the number(and severity) of cases.In one series of 220 cases in Sabah, Malaysia, found no seasonal variation in the occurrence of cases [10,11].The single most important risk factor for developing severe melioidosis is diabetes mellitus. Other factors include thalasaemia, haematological conditions, HBE trait, malignancy, steroids therapy, immune deficiency disease, alcoholism, drug abuse, renal disease/failure, COAD, penetrating injury, and near drowning. The mode of infection is by respiratory or cutaneous route [12,13].Clinical presentations of melioidosis, being the fulminant end of clinical spectrum, with abscesses throughout both lungs and in in many organs[14].It has also been classified as acute, sub-acute or chronic[15].The diagnosis of the disease depends on culture of pathogen from blood, urine, sputum, pus, bronchial, bone joint aspirate.The rate of successful culture is increased if sputum, throatswabs, ulcer or skin lesions swabs, and rectal swabs are placed into Ashdown's medium, a gentamicin- containing liquid transport broth that results in the selective growth of *B.pseudomallei*[16,]. *B.pseudomallei* can identified by combining the commercial API 20NE or 20NE biochemical kit with simple screening system involving the Gram stain, oxidase reaction, typical growth characteristics, and resistance to certain antibiotics[17].Antibiotics of choice being the β-lactam, especially ceftazidime, imipenem,meropenam,pipercillin,amoxicillin-clavulanate,ceftriaxone,and cefotaxime, with various degrees of bactericidal activity. Initial intensive intravenous therapy of 10 to 14 days, and eradication therapy minimum of three months or more is required[18].The paper reviews the current literature,clinical presentations, and treatment of melioidosis.

II.HISTORY

The name melioidosis is derived from Greek "melis" meaning" a distemper of asses" with suffixes-oid meaning "similar to" and –osis meaning "a condition" that is, a condition similar to glanders[1] Melioidosis was first discovered in Burma (now Myanmar) by Whitmore and Krishmaswami in 1912.After its discovery in Burma, melioidosis was documented in humans and animals in Malaysia and Singapore from 1913 and then Vietnam from 1923 and Indonesia from 1929[19-21].Thailand had reported the highest number of cases[22-24], with an estimated 2000 to 3000 cases of melioidosis each year[25].Melioidosis is also common in Malaysia and

Singapore [26-28].Other countries in the region where melioidosis is recognized in human and animals include China(especially Hong Kong), Taiwan, Brunei, Veitnam, and Laos[29-33].Melioidosis is also likely to occur in Cambodia and Philippines [21,25,34]. Melioidosis has been increasingly recognized in India, although reports that some of the "Plague" scares of 1994 may have been cases of melioidosis have been disapproved[35-36].Cases have been reported from Sri Lanka, Bangladesh, and Pakistan[21].Despite the early documentation of melioidosis in Burma and Indonesia, recent cases had not been reported from Indonesia until after Asian tsunami[37]. Cases of melioidosis have also been documented from Papua New Guinea, Fiji, and New Caledonia, but the extent of the indemnity in the Pacific islands remains to be defined[38]. Melioidosis cases are increasingly being documented from outside the classic endemic region of Southeast Asia, Australasia, the Indian subcontinent, and China. These include sporadic human or animal cases or environmental isolates of *B.pseudomallei* from Middle East, Africa, the carribean, and Central and South America. Although some of these reports are from incorrect species diagnosis, other are confirmed making the endemic limitations of melioidosis very unclear [21]Sporadic cases and occasional case clusters have recently occurred in Brazil and elsewhere in America[39].Despite recent cases from Madgascar, the true extent and magnitude of the presence of *B.pseudomallei* in Africa remains entirely unknown[40].Global warming may well result in expansion of endemic boundaries of melioidosis [41].

The two locations where melioidosis is arguably the most important single bacterial pathogen for humans are northeast province in Thailand and the Top End of the Northern Territory of Australia.In northeast Thailand,20% of community- acquired septicemic cases are caused by melioidosis,which accounts for 39% of fatal septicemias and 36% of fatal community acquired pneumonias[23,31].In the Top End of Northern Territory, melioidosis has been the most common cause of fatal community-acquired bacteremic pneumonia[42].

In addition to endemic melioidosis, there are several documented situations where melioidosis became established in nontropical locations. In France, in the 1970s animals in a Paris Zoo, with spread to other Zoos and equestrian clubs[19]. In addition to fatal animal and human cases, there was extensive soil contamination persisting for some years. *B.pseudomallei* was considered likely to have been introduced by importation of infected animals. A cluster of cases occurred over 25- year period in southwestern Western Australia (31^{o}.S),involving animal cases and one human infection in a farmer. Ribotyping of farm animal and human isolates and one isolate from the soil showed identical patterns [43]This supports the suggestion of clonal introduction of *B.pseudomallei* into this this temperate region, probably via infected animal, with environmental contamination, local dissemination and persistence over 25 years[41].

III. PATHOGENESIS

Worldwide studies have shown that most infection with *B.pseudomallei* is asymptomatic [44]. In northeast Thailand, most of the rural population is seropositive by indirect hemaggultination(IHA),with most seroconversion occurring between 6 months and 4 years of age[45]. Although the melioidosis occurring in all age groups, severe clinical disease such as septicemic pneumonia is seen mostly in those with risk factors such as diabetes, renal disease, and alcoholism[44]. In addition to 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 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 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, humans, and the environment that virulence differs among *B.pseudomallei* isolates [46].the importance of this variation in virulence in determining clinical aspects of melioidosis remain unclear. Molecular typing that shows clonality of isolates in animal and human clusters has revealed that same outbreak strain can cause different clinical presentations, with host factors being most important in determining the severity of disease [47].Whole genome sequencing and subsequent molecular studies have shown that *B.pseudomallei* has two chrosomes, multiple genomic islands that are variably present in different strains and have a great propensity for horizontal gene transfer[48].Further studies are required to unravel the global phylogeny and evolutionary history of *B. psedumallei* and related species and to determine which genes or gene clusters may be critical for pathogenesis and disease presentation and outcome [49]. *B.pseudomallei* is a facultative intracellular pathogen that can invade and replicate inside various cells, including polymorphonuclear leukocytes and macrophages and some epithelial cells[50].Animal models have been unable to confirm a clinically relevant exotoxin for *B.pseudomallei*[51].However resistance to human serum (conferred by lipopolysaccharide[LPS])[52],and the ability of *B.pseudomallei* to survive intracellularly (conferred in part by capsular polysaccharide) appear to be critical in the pathogenesis of melioidosis[53-55].Type III secretion system in *B.pseudomallei* have also been found to be important in cell invasion and intracellular survival[56-57].

Quorum sensing may play an important role in many aspects of virulence of *B.pseudomallei* including cell invasion, cytotoxicity and antimicrobial resistance[41,58-59].Other putative virulence factor candidates include flagella, type IV pili and other adhesion, a siderophore , and secreted proteins such as hemolysin lipases, and proteases[58]. Intracellular survival in human *B.pseudomallei* in human and animal hosts is likely to explain the ability for latency. After internalization, *B.pseudomallei* escapes from endocytic vacuoles into cell cytoplasm, and induction of actin polymerization at one bacterial pole leads to membrane protrusions, with cell-to-cell spread involving these actin tails [57-59].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 glycocalyx structures to form biofilm-encased micro colonies that are also relatively antibiotic-resistant [60].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 in not established. In Thailand, there was an association of severe melioidosis with tumor necrosis factor (TNF).-α gene allele 2, which is linked to high constitutive and inducible production of TNF-α[61]. However, in a mouse model of melioidosis,neutralization of TNF-α or interleukin(IL)-12 increased susceptibility to infection in vivo, and interferon-ɣ (IFN-ɣ) was found to be important for survival, with mice treated with monoclonal anti-IFN-ɣ dying more quickly[62].

A role for Toll- like receptors in the innate immune response in melioidosis has been proposed[63].There are therefore, important host protective mechanisms against *B.pseudomallei* in cytokine responses as well as potentially detrimental ones, with timing of cytokine release and the balance, between pro-and anti-inflammatory responses likely to determine the severity of disease and outcome of infection[,58,64]The extent to which host polymorphism 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[41].

The most important risk factors are diabetes, alcohol excess and renal disease [65-67].In Thailand, the adjusted odds ratio for diabetes and renal disease (chronic renal impairment or renal or ureteric calculi) in cases of melioidosis versus controls 12.9(95% confidence interval(CI),5.1 to 37.2) and2.9(95% CI,1.7 to 5.0),respectively[67].Other risk factors for melioidosis include chronic lung disease(including cystic fibrosis),thalassemia(odd ratio in Thailand,10.2;95% CI,3.5 to 30.8),malignancies, steroids therapy, iron overload, and tuberculosis[41,67]. Severe disease and fatalities are uncommon in those without risk factors, who are diagnosed and treated early, with only one death in 51 patients without risk factors in one study[41,66].Risk factors are less common in children than in adults[41,68,69].Evidence suggests that there may be a predisposition to melioidosis in those with diabetes, alcohol excess, or chronic renal disease, which may reflect impairment of their neutrophil and other phagocytic cell functions, such as mobilization, delivery, adherence, and ingestion[66,70].Melioidosis has also been described in chronic granulomatous disease[71].

IV. CLINICAL MANIFESTATIONS

Clinical presentations of melioidosis has been described as the fulminant end of the clinical spectrum, with abscesses throughout both lungs, and in many organs [14].At the other end of the spectrum are asymptomatic infections and localized skin ulcers or abscesses without systemic illness. Howe and colleagues have classified melioidosis as acute,subacute,and chronic[15].The infectious Disease Association of Thailand has summarized 345 cases in these categories[41,65,22].include:(a)Multifocal infection with septicemia (45% of caes,87 % mortality(b)Localized infection with septicemia (12% of cases,17% mortality)(c) Localized infection (42% of cases,9% morality)(d)Transient bacteremia (0.3%)Most recent bacteremia and overall, mortality have been respectively,60% and 49%,46% and 19% in Australia. And 43% and 39% in Singapore [41, 45 ,66, 21] Researchers in Sabah,Malaysia,reported 75% mortality in septicemic melioidosis[11].

In Australia pneumonia is the commonest clinical presentation of patients with melioidosis in all studies, accounting for around half of cases. Secondary pneumonia after another primary presentation occurs in around 10% of cases. Acute melioidosis pneumonia has a 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[41]. Septicemic 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 caseous necrosis and multiple metastatic abscess formation seen at autopsy [41]. Nonsepticemic patients with pneumonia and some with septicemic pneumonia have a more predominant cough, with productive sputum and dyspnea, and their chest radiographs show discrete but progressive consolidation in one or both lobes. In endemic regions, acute pneumonia with upper lobe consolidation warrants consideration of melioidosis, although lower lobe infiltrates are also common[41].

In 12% of cases in northern Australia, patients present with chronic melioidosis defined as illness with symptoms for longer than 2 months duration on presentation. Many of these patients have features mimicking

tuberculosis, with fevers, weight loss, productive cough (sometimes with hemoptysis),and classic upper lobe infiltrates, with or without cavitation on chest radiographs. In these patients, disease can be remitting and relapsing over many years, sometimes with a misdiagnosis of tuberculosis. Although acute deterioration with septicemia may occur, mortality in this group is low [41].

Until recently it was thought that a colonization state did not exist for *B.pseudomallei*, with presence in sputum or throat always reflecting disease. However, it has recently become evident that *B.pseudomallei* can both colonize always and cause disease in patients with cystic fibrosis(CF) and bronchiectasis. The similarity to infection with *B.cepecia* complex in CF is of concern, given the association of *B.cepecia* complex with more rapid deterioration in lung function. Furthermore, likely transmission of *B.pseudomallei* between two siblings with CF has been reported[41,72]. Patients with CF traveling to melioidosis-endemic locations should be warned of the risk of melioidosis, which could be considered if they become sick after returning [41].

It is common for patients to present with skin ulcers or abscesses [73].Occasionally they present with septic arthritis or osteomyelitis, or one of these can develop after the patient has presented with another primary diagnosis, usually pneumonia. Also well recognized, whatever the clinical presentation, are diseases in internal organs, especially in spleen, kidney, prostrate and liver. Where available, abdominopelvic computed tomography (CT) scanning is useful in all melioidosis patients to detect internal abscesses [41].

These have been noted between Thailand and tropical Australia. First, suppurative parotitis accounts for up to 40% of melioidosis in children in Thailand [68, 74],but is very rare in Australia.Second, prostrate melioidosis is well recognized but uncommon, except in Australia, where routine abdominopelvic CT scanning of all melioidosis cases has shown prostrate abscesses present in 18% of all male patients with melioidosis[66].Some were incidental in patients presenting with pneumonia or septicemia, but a primary genitourinary presentation was common with fever, abdominal discomfort, dysuria, and sometimes diarrhea and urinary retention. Third neurological melioidosis accounts for 4% of cases in northern Australia, with distinctive clinical features being brain stem encephalitis, often with cranial nerve palsies(especially the seventh nerve),together with peripheral motor nerve),together with peripheral motor weakness, or occasionally just flaccid paraparesis alone.The CT scan is often normal, but dramatic changes are seen on magnetic resonance imaging(MRI),most notably a diffusely increased T2-weighed signal in midbrain, brain stem, and spinal cord[41,75].Direct bacterial invasion of the brain and spinal cord occurs with melioidosis encephalomyelitis[41,76].

Neurological melioidosis is occasionally seen outside Australia, although mostly macroscopicbrain abscesses [41, 22, 77]. Unusual foci of melioidosis infection described in case reports or case series include mycolic aneurysms, lymphadenitis resembling tuberculosis, mediastinal masses, pericardial collections, and abnormal abscesses.

It has long been recognized that *B.pseudomallei*, like tuberculosis, has the potential for reactivation from a latent focus, usually in the lung-hence the concern of the "Vietnamese time bomb" in returned soldiers. Latent periods from exposure to *B.pseudomallei* in an endemic region to onset of melioidosis in a nonendemic region have been documented as long as 62 years [78].However, cases of reactivation *B.pseudomallei* appear to be very uncommon, accounting for only 3% of cases in northern Australia.The vast majority of cases of melioidosis occur in the monsoonal wet seasons of various endemic regions, supporting the concept that endemic areas most patients with melioidosis have recent infections that appear with acute illness.Reactivation of melioidosis has been associated with influenza, other bacterial sepsis, and development of known melioidosis risk factors such as diabetes. It remains unknown what proportion of asymptomatic seropositive people actually have latent infection with potential for reactivation [41].

V.DIAGNOSIS

There are no reliable pathognomonic features of acute or sub-acute melioidosis. Other infections including tuberculosis and typhoid fever are commonly confused with melioidosis. The commonest clinical presentation of the disease in northern Australia and Southeast Asia where the infection appears to be commonest is septicemia with or without pneumonia[75].The majority of severe infections occur in patients with contributory co-morbidity such as uncontrolled diabetes mellitus, chronic renal failure, alcoholic liver disease or chronic lung disease [42].A history of contact with soil, water and percutaneous inoculation is also a risk factor of melioidosis [7].

A definitive diagnosis of melioidosis requires a positive culture of *B.pseudomallei*. Melioidosis must be considered in a febrile patient in or returning from endemic regions to enable appropriate samples to be tested. *B.pseudomallei* readily grows in commercially available blood culture media, but it is not unusual for laboratorities in nonendemic locations to misidentify the bacteria as *Pseudomonas* species, especially because some commercial indectification syatems are poor at identifying *B.pesudomalli*[[79].Culture from nonsterile sites increases the likelihood of diagnosis but can be problematic. The rate of successful culture is increased if sputum, throat swabs, ulcer or skin lesion swabs, and rectal swabs are placed into Ashdown's medium, a gentamicin-containing liquid transport broth that results in the selective growth of *B.pseudomallei* [15].

B.pseudomallei can identified by combining the commercial API 20NE or 20E biochemical kit with a simple screening system involving the Gram stain, oxidase reaction, typical growth characteristics, and resistance to certain antibiotics[16]. There are a variety of locally developed antigen and DNA detection techniques used in endemic regions for early identification *of B.pseudomallei* in culture media and patient blood or urine but these are not yet widely available[16].An indirect hemaggultination test(IHA). Various enzyme-linked immunosorbent assays(ELISAs),and other serological assays are available[41,80].In endemic areas, their usefulness is limited by high rates of background antibody positivity.In acute septicemic melioidosis,IHA,and ELISA are often initially negative, but repeat testing may show seroconversion.A positive IHA, or ELISA in a tourist returning from a melioidosis –endemic region is useful in supporting the possibility of melioidosis, but definitive diagnosis still require a positive culture[80].

VI.TREATMENT

B.pseudomallei is characteristically resistant to penicillin, ampicillin. first-and second generation cephalosporin's, gentamicin, tobramycin, and streotomycin. Before,1989,"conventional therapy" for melioidosis consisted of a combination of chloramphenicol, sulfamethoxazole-tromethoprim,doxycycline,and sometimes kanamycin, given for 6 weeks to 6 months[65].However ,there were reports of the successful use of sulfametoxazole-trimethoprim alone and tetracycline or doxycycline alone.These conventional antibiotics are bacteriostatic rather than bacteriocidal, and in studies have shown various combinations to be antagonistic [41].Subsequent studies have shown *B.pseudomallei* to be susceptible to various Beta-lactam antibiotics ,especially, ceftazidime, imipenem, meropenem,pipercillin, amoxicillin-clavulanate, ceftriaxone, and cefotaxime, with various degrees of bactericidal
activity.

Initial intensive therapy minimum of 10 to 14 days include ceftazidime,(50mg/kg, up to 2 g, every 6 hourly or meropenem(25mg/kg up to 1 g) every 8 hour, or imipenem(25 mg/kg up to 1 g) every 6 hour. Any one of the three may be combined with sulfamethoxazole-trimethoprim (40/8 mg/kg up to 1600/320 mg) every 12 hour (recommended for neurological, cutaneous, bone and prostatic melioidosis).
Eradication therapy minimum of 3 months Sulfamethoxazole (40/8mg/kg up to 1600/320) every 12 hour with or without Doxycycline(2.5mg/kg up to 100 mg) every 12 hour[41].

VII. CONCLUSION

Septicemic melioidosis has high mortality, high clinical suspicion is required and appropriate empirical therapy should be instituted.

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