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CHALLENGES IN THE DIAGNOSIS AND TREATMENT OF MELIOIDOSIS: A REVIEW

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ABSTRACT

Melioidosis is a disease of global concern which is caused by a gram-negative bacterium, *Burkholderia pseudomallei*. About 412,000 human infections of Melioidosis is estimated each year globally, from which nearly 89,000 people die (infection to death ratio = 5:1 nearly). The Centers for Disease Control and Prevention (CDC) has listed it as a potential bio-threat due to its undetected incidence and diagnosis with diverse clinical manifestations with a general lack of adequate diagnostic capabilities in most of the areas along with very high antibiotic-resistance of *B. pseudomallei*. It is a rarely reported disease probably due to the lack of awareness among the health-care professionals and public. It often mimics tuberculosis or other diseases during the initial presentation and is thus misdiagnosed due to the similarity in presenting signs & symptoms. Another concern with the disease is its clinical presentation, differential diagnosis and treatment. Melioidosis is a highly under-diagnosed disease but starting pharmacological treatment by suspecting the disease based upon clinical presentations and by ruling out all possible diseases can be life saving for the patients. This review provides an outline of clinical presentation, differential diagnosis and treatment. It also highlights the challenges faced in diagnosis by way of difficulty in confirmation by culture test due to the requirement of sophisticated laboratory & skilled technicians and methods to overcome high antibiotic resistance.

KEYWORDS: Melioidosis, Whitmore disease, Burkholderia pseudomallei, Diagnosis, Antibiotic resistance, bio-threat.

INTRODUCTION

Melioidosis is also known as Whitmore disease.[1] The first description and report of Melioidosis was done in 1911 when Indian bacteriologist C.S. Krishnaswami, under the guidance of pathologist Whitmore described a 'Glanders like' disease seen among the morphine addicts in Rangoon, Burma.[1] The causative agent for Melioidosis is a gram-negative bacterium, *Burkholderia pseudomallei*, a motile, aerobic, non-spore forming bacillus.[1-4] The Centers for Disease Control and Prevention (CDC), United States of America has listed Melioidosis as '*Bioterrorism Agents/Diseases*' under '*Emergency preparedness and response*' section of its website.[2-4] Nearly 412,000 human infections of Melioidosis is estimated[5] each year worldwide, from which nearly 89,000 people die (infection to death ratio = 5:1 nearly). It often mimics tuberculosis in the initial presentation and treatment with anti-tubercular therapy is common.[1-7] The bacteria thrive in tropical climatic Zones and is an endemic in the South-East Asia and South Asian region and though cases are likely to be present in India too but are under-reported.[6, 8]

Signs and Symptoms

There is a general lack of awareness in most of the countries endemic for this disease and its signs and symptoms varies with the site of infection. CDC lists the following signs and symptoms: for ***Localized Infection***: localized pain or swelling, fever, ulceration and abscess; for ***pulmonary infection***: cough, chest pain, high fever, headache and anorexia; for ***bloodstream infection:*** fever, headache, respiratory distress, abdominal discomfort, joint pain and disorientation; and for ***disseminated infection:*** fever, weight loss, stomach or chest pain, muscle or joint pain, headache and seizures.[4 9] The disease is mainly reported from Australia and Thailand and found in the USA and Europe as well.[9] It is one of the emerging infectious diseases to cause morbidity and mortality in South-East Asia, Northern Australia and other tropical regions.[8,10]

Epidemiology

There are some drawbacks in collecting epidemiological data about this disease because of the disease under-diagnosis, since it requires sophisticated laboratory facilities.[10,11] Melioidosis mainly occurs in the persons who are regularly in contact with soil and contaminated water, with a higher risk amongst those involved in farming, and also in travelers.[11] There is a frequent increase in the disease occurrence during rainy season. Some studies suggested that the transmission occurs through inoculation, inhalation and ingestion.[12] Transmission mainly occurs through inhalation by aerosols and cases were particularly reported in helicopter crewmen, during the Vietnam War, when helicopter crewmen of United States (US) became infected in Vietnam and since 1973, following the withdrawal of US forces from Vietnam, there had been 343 reported cases of Melioidosis resulting in 36 deaths.[13] Melioidosis in many cases formed a latent infection which only recrudesced a significant time after return to the US, giving rise to the name "time-bomb disease" among ex-servicemen.[13]

There were confirmed cases in United States reported from travelers and immigrants coming from the endemic areas.[2-4, 14,15] Melioidosis is under-reported in 45 Countries. studies suggests that Melioidosis is endemic in future and 34 other countries have not reported a single case till date.[2-4, 13,14]The disease was first detected in 20th century in Aruba, a tiny Dutch Caribbean island off the coast of Venezuela, in animals like sheeps, goats and pigs. The organism which is responsible to cause the disease is also considered as a potential biological warfare agent.[3,14]

Epidemiological data shows one fifth of the Melioidosis patients are community acquired cases of septicaemia where the mortality rate is around 50%.[15]The first case of Melioidosis was reported in a horse.[16] The main issue in eradication of organism is due to improper diagnosis since the disease shows the similar clinical manifestation associated with Tuberculosis, Pneumonia and hence in clinical practice it is difficult to diagnose.[15] Under-diagnosis is the factor that contributes to the irrational use of antibiotics which further contributes to resistance of antibiotics, unnecessary increase in health care cost, and influences the increase in morbidity and mortality rate of the disease as well.[2,4,15]

Several cases were reported from North Brazil, with highest rainfall in the area, and the disease was seen more in adventure travellers, tourists, military personnel, construction workers, sanitary workers and people who are mostly in contact with contaminated water or soil, or had spent time with endemic areas.[1,3,4,14-16] Majority of severe infections was reported to occur in patients with diabetes, renal diseases, pulmonary diseases (mainly COPD), thalassemia patients, patients having any malignancy (mainly haematological malignancies) and immunosuppressive disease, retrovirus infections, collagen vascular disease, cystic fibrosis and alcoholics, mainly kava (a Hawaiian drink) consumers.[2,4,17]

Melioidosis has been nicknamed the ‘‘great mimicker’’ due to its diverse clinical manifestations, ranging from asymptomatic latent, to chronic localized, to acute septicaemia forms.[18] There are rarely any cases where the transmission of disease was reported from person to person or animal to person and hence not listed as a risk factor by the CDC in these categories but chances of such spread cannot be ruled out as it can be easily spread by aerosols or by coming in contact with or ingesting contaminated soil or water as well.[4,18] The disease is reported to be endemic in South-East Asia, Papua New Guinea, much of the Indian subcontinent, southern China, Hong Kong and Taiwan and is considered highly endemic in northeast Thailand, Malaysia, Singapore, and northern Australia.[4,19] Sporadic cases have been reported among residents of or travelers to Aruba, Colombia, Costa Rica, El-Salvador, Guatemala, Guadeloupe, Honduras, Martinique, Mexico, Panama, Venezuela and many other countries in the Americas, as well as Puerto Rico.[4,18-30]

This organism exists in soil and water in Melioidosis-endemic regions of the tropics, and infection is acquired through bacterial inoculation, inhalation, and aspiration. Clinical manifestations of infection are very broad ranging, but the most frequent presentation is that of a septicaemia illness associated with bacterial dissemination to distant sites.[27-33] One fifth of cases in North-East Thailand occur in children. Overall mortality is 50% in North-East Thailand (35% in children) and 19% in Australia.[32,33] Many cases were found to be reported from Thailand (around 2000-3000 cases) every year and also commonly found to be reported from Malaysia and Singapore in 1913. It was found both in animals and humans.[34] Melioidosis first originated in Burma and reporting has been done in Vietnam in 1925 & in Indonesia from 1929.[32] Melioidosis was also reported in other counties like China, Taiwan, Cambodia, Philippines, and data showed increasing rates in India. Most of the cases have been reported in South-East Asia, Australia, Indian subcontinent, and China Some cases also reported in Sri Lanka, Bangladesh and Pakistan.[4,32] In North-East 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.[4, 11, 32, 35]

DIAGNOSIS

Apart from the clinical presentation, the organism can be identified by culture, molecular identification and by serodiagnosis. Imaging like X-ray can help in diagnosing the pulmonary spread of disease. The different methods for diagnosis and confirmation of the disease are as follows:

A. Differential diagnosis of Melioidosis

It includes pyrexia of unknown origin (PUO), acute respiratory distress syndrome and acute septicaemia.

Other conditions including pneumonia, acute suppurative lesions, chronic granulatomous lesions, septic arthritis, osteoarthritis, osteomyelitis and mycotic aneurysm[36] CRP (C-reactive protein) and radiological chest X-ray should be carried but it should not show the positive sputum for tuberculosis. Diabetic patients with Melioidosis shows leucocytosis and changes in urea and creatinine level.[37]

B. Laboratory tests

Culture for *B. Pseudomallei*

B. pseudomallei is a non-fastidious species, able to grow on minimal media supplemented with a wide range of carbon and nitrogen sources. Non-selective diagnostic laboratory media such as blood agar is sufficient for isolation of *B. pseudomallei* from blood and other sterile fluids. However, selective media such as Ashdown's selective agar (ASA) are required to isolate *B. pseudomallei* from non-sterile sites.[37,38] ASA is used for testing the non-sterile sites for confirmation of the organism, the incubation period involves at least for 24 hours. And most importantly other media is also used to differentiate and recover the *B. pseudomallei* from another gram-negative bacterium (*B. cepacia* and *Pseudomonas aeruginosa*) is BPSA. Selection of media depends upon the specimens used for the test. Suitable specimens used for the above tests are[38] blood inflammatory exudate, sputum, and autopsy specimens.[38] Sensitivity of the test depends upon the type of media used, the type specimen used for the test, storage conditions of the culture and enrichment of the media.[38] Almost 20% of the tests are misidentified for other bacterium and thus, it is important to confirm the causative bacterium. The test used to confirm the bacterium is agglutination of antisera nucleic acid amplification.[38]

Special considerations

It is important that laboratories correctly identify the species present in a specimen to avoid false positive and false negative etiological diagnoses. In some locations, up to Usually, 98% clinical isolates can be identified easily using standard clinical laboratory methods. *B. pseudomallei* can be differentiated from *B. cepacia* by supplementary test such as nucleic acid amplification or gas-liquid chromatography for bacterial cell wall fatty acid methyl esters. Both these methods are known to generate equivocal results that do not distinguish some clinical *B. pseudomallei* strains from *B. cepacia.*[38]

Conventional biochemical tests

98% of the tests identify the strain by substrate utilization panels. Presence of oxidative reaction, antibiotic susceptibility, gram stain appearance and colony appearance makes it easier to predict the confirmation of strain.[38-40]

Predictive values

Prolongation of the incubation period of the test is necessary to identify and confirm the strain. Substrate utilization technique with negative result doesn't exclude the strain (*B. pseudomallei*).[38]

Suitable test criteria

It involves the results with specific characters, gentamycin resistant amoxicillin-clavulanic acid sensitive, gram negative bacilli, staining, safety pin appearance ADH, oxidative and gelatinase positive.[38-40]

Some kits available for identification *of B. pseudomallei*[38]

Identification of *B. pseudomallei* is done by using various substrate utilization kits. The 20NE and AP120E listeria and Macrobact 24 are used in Australia.[38]

Molecular identification

The identification of *B. pseudomallei* is done by the Nucleic acid amplification test.[38]

Serodiagnosis

There are different Sero-diagnostic methods available for the identification of the strain. The tests include ELISA and indirect Hemagglutination test. These tests contribute in easy interpretation of the results.[38,41]

Suitable test criteria

ELISA or Hemagglutination should be positive.[38]

Diagnostic criteria

The characteristic feature of the gram-negative bacillus of the specimen should hold the following features:Gram negative, oxidative positive, polymyxin resistant and PCR positive, *B. pseudomallei* antibody positive, an increased *B. pseudomallei* antibodies by ELISA.[38-41]

PHARMACOLOGICAL TREATMENT APPROACHES

Before selecting pharmacological approaches to treat the Melioidosis, proper diagnosis must be carried out since this infectious disease mimics as Tuberculosis, Malaria or Pneumonia because of presence of certain similar characteristic features.[42,43] Treatment approaches are divided into two phases which involves minimum intensive phase and eradication phase.

A. Intensive phase

Duration of intensive phase involves up to few weeks and duration of eradication phase involves months. The ideal antibiotic used to treat the Melioidosis is intravenous ceftazidime because of its rapid action as a bactericidal[44] or carbapenem (meropenem or imipenem) where the duration of the treatment should be at least for 10 days[45] and followed by oral drugs with trimethoprim sulfamethoxazole (TMP-SMX) as a monotherapy or combined therapy with doxycycline continued at least for 12 to 20 weeks in eradication phase with lower risk of reoccurrence.[46]

B. Eradication phase

This phase mainly involves the prevention of diseases from relapse. Minimum duration of this therapy involves around 12 weeks, co-trimoxazole is the first choice for the treatment of disease in this phase if patient has no history of known allergies. In case shows that any documented allergies or patient is intolerant to the first line drug, co-amoxiclav is the second line drug with a ratio of 4:1. Dose is adjusted with respect to body weight.[47]

GUIDELINES FROM DIFFERENT COUNTRIES

Northern Territory of Australia Guidelines

The guidelines from the northern territory of Australia suggest that the first phase of the IV antibiotics treatment should be continued at least for 14 days of duration. If the patient persists with severe infection, treatment duration can be extended up to 4 weeks. The period of eradication phase should be minimum of three months. Some other parts of the world use combined therapy of drugs like Doxycycline with TMP-SMX is used in eradication phase. This regimen also holds strong supportive evidence with previously conducted Studies Initial therapy started with Ceftazidime 2 gm and in paediatrics 50 mg/kg up to 2 gm IV hourly treatment continued at least 14 days Meropenem 1 gm in adults and in paediatrics, 25 mg/kg IV 8 hourly treatment should be continued for at least for 14 days. Granulocyte colony stimulating factor (GCSF) 300 µg IV is indicated in patients with septic shock, continued for 10 days. and this therapy is contraindicated in patients with acute coronary syndrome and increased WBC count. [38]

The Centers for Disease Control and Prevention (CDC)

CDC recommends the following[4]

A. Intravenous therapy

Ceftazidime administered every 6-8 hours **or** Meropenem administered every 8 hours.

B. Oral antimicrobial therapy

Trimethoprim-sulfamethoxazole taken every 12 hours **or** Doxycycline taken every 12 hours**.**

The type of infection and the course of treatment will impact long-term outcome. Treatment generally starts with intravenous antimicrobial therapy for 10-14 days, followed by 3-6 months of oral antimicrobial therapy.

IMMUNE MODULATION THERAPY TO OVERCOME RESISTANCE[48-51]

A barrier of high resistance made difficult to treat Melioidosis reoccurrence. To overcome this some recent research suggest that conjunctive therapy, which involves immunomodulation by inhibiting COX-2 which reduces the production of PGE-2. It is is an effective therapy to overcome the resistance. This made researchers to identify some drugs to overcome this problem. The identified Non-steroidal anti-inflammatory drug Tolfenamic acid (TA) marketed in US as an indication for Migraines. On exposure with Tolfenamic acid with sub therapeutic treatment with ceftazidime in mice, results revealed an increase in cell viability in vitro with TA and could reduce both COX-2 expression and PGE2 production while also decreasing NFκB activation in infected patients.

Recent study supports of immune modulation therapy by inhibiting cyclooxygenase-2 inhibitor (COX-2) to inhibit the synthesis of prostaglandins (PGE2) which is considered as the most effective post exposure therapy for Melioidosis is initially of drug resistance can be decreased by immunomodulation therapy. Immunomodulation can be an additional therapy used to increase the effectiveness and decreases the antibacterial dose requirement. Neurological Melioidosis initially treated with meropenem IV and dose is increased to twice to 2 gm and in paediatrics, it is 50mg/kg up to 2 gm given every 8 hourly.[51-53]

MONITORING PARAMETERS

Some of the monitoring parameters which are taken into the account during the treatment approaches are urea, creatinine, electrolytes, Liver Function Tests, WBC count (eosinophil), CRP and dosage adjustment[46,53] is required in patients with renal impairment.

CHALLENGES DUE TO ANTIBIOTIC RESISTANCE

Selection of appropriate antibiotics is the most challenging task in clinical practice to treat Melioidosis because the bacillus has high resistance to antibiotics. The gram-negative bacterium, *B. pseudomallei*, with its characteristic feature, that is naturally resistant to number of most of the antibiotics, namely cephalosporin, penicillin, aminoglycosides and the mechanism involved in the antibiotic resistance includes enzymatic inactivation,[52, 53] target deletion and efflux from the cell and mediated by chromosomally encoded genes. Genetic mutation in the beta lactamase lead to ceftazidime, amoxicillin- clavulanic acid resistance and loss of protein binding sites for penicillin also leads to ceftazidime resistance.[54]

Expression of some efflux pumps namely BpeAB-OprB, a multidrug efflux pump in *Burkholderia pseudomallei*,, cause trimethoprim and trimethoprim-sulfamethoxazole resistance. There are reports which support[55-57] high resistance to antibiotics for *B. pseudomallei*. The antibiotics namely: amoxicillin, ticarcillin, ceftoxitin, cefsulodin, aztreonam, and aztreonam, ceftazidime show resistance at high concentrations with MIC around 64 mg/L. Studies have shown that doxycycline and minocycline are most accepted antibiotics and considered as alternative treatment in ceftazidime to treat Melioidosis. Most of the infectious diseases are treated with doxycycline and minocycline and also these antibiotics show less resistance to *B. pseudomallei* and because of this reason these antibiotics are used in treatment of localised and severe Melioidosis. It has been

recommended by EMEA as combined therapy with imipenem or meropenem in severe cases of Melioidosis. It has been used as post exposure prophylaxis.

The resistance for erythromycin, clindamycin, aminoglycosides (gentamycin, tobramycin, netilmicin, amikacin) is due to presence of multidrug efflux system in *B. pseudomallei*, and this specific character holds for both aminoglycosides and macrolides. The overall resistance of antibiotics for Melioidosis is high and in conclusion imipenem, ceftazidime, piperacillin tazobactam and doxycycline are most effective drugs are currently recommended to treat Melioidosis and piperacillin/tazobactam can be used as an alternative therapy for meliodosis.[55-57]

PREVENTION

The population who are at risk like diabetes, renal disease and other risk factors, should avoid contact with contaminated water and soil.[2-4] Persons who are working in the paddy fields should wear protective gears to avoid infection through skin.[2-4] Health care professionals who are in regular contact with infected patients should use masks and gloves to prevent the infection. The migrants from the endemic areas should undergo diagnosis for the bacterium.[2-4, 42, 43, 45]

CONCLUSIONS

Melioidosis is often mistaken to be Tuberculosis, Malaria or Pneumonia due to similar presenting signs & symptoms as it is very difficult to diagnose due to limitations of laboratories and cultures in all the areas endemic for Melioidosis. The differential diagnosis of any pyrexia when the origin is unknown should be considered for possible Melioidosis and diagnosis should be confirmed with the help of blood, urine, sputum, imaging or skin-lesion testing. Sometimes the laboratory findings cannot provide sufficient evidence for confirming Melioidosis, but starting pharmacological treatment by suspecting Melioidosis based upon clinical presentation of signs & symptoms and ruling out all other possible diseases infection in the patient (diagnosis by exclusion) can be life saving for the patients, especially in the areas where the disease is common or if the patient has recently travelled to endemic areas for Melioidosis.

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CONFLICT OF INTEREST

No conflict of interest.

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