

Original Article

Osteoarticular and soft-tissue melioidosis in Malaysia: clinical characteristics and molecular typing of the causative agent

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Abstract

Introduction: Melioidosis involving bone, joints, and soft tissue is rare and reported usually following dissemination of disease from infection elsewhere in the body; to a lesser degree, it can also be reported as the primary manifestation of melioidosis.

Methodology: The orthopedic registry at Hospital University Sains Malaysia from 2008 until 2014 was retrospectively reviewed and was followed by molecular typing of *Burkholderia pseudomallei*.

Results: Out of 20 cases identified, 19 patients were confirmed to have osteoarticular and/or soft-tissue melioidosis. The majority of the patients were males (84%), and 16 patients had underlying diabetes mellitus with no significant estimated risk with the disease outcomes. Bacterial genotype was not associated with the disease as a risk. Death was a significant outcome in patients with bacteremic infections (p = 0.044). Conclusion: Patients with lung or skin melioidosis require careful treatment follow-up to minimize the chance for secondary osteoarticular infection. Human risk factors remain the leading predisposing factors for melioidosis. Early laboratory and clinical diagnosis and acute-phase treatment can decrease morbidity and mortality.

Key words: bone; joint; soft tissue; *B. pseudomallei*; MLST.

J Infect Dev Ctries 2017; 11(1):28-33. doi:10.3855/jidc.7612

(Received 31 August 2015 – Accepted 08 February 2016)

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Introduction

Musculoskeletal melioidosis is rare but not uncommon; septic arthritis and osteomyelitis are usually secondary infections that commonly occur following bacterial dissemination from infection elsewhere in the body. To a lesser extent, septic arthritis or osteomyelitis can be the primary manifestation of melioidosis. The commonest musculoskeletal melioidosis presentations are soft-tissue involvement, followed by osteomyelitis, septic arthritis and, less commonly, spine infection [1].

In endemic countries, melioidosis should be suspected in diabetic patients with febrile illnesses presenting with multiple subcutaneous lesions, respiratory failure, or tuberculosis-like radiological changes. Routine diagnosis of melioidosis is made by the cultivation of *B. pseudomallei* from clinical specimens. Indirect hemagglutination assay (IHA) is widely performed for epidemiological purposes. Molecular techniques have been used for rapid diagnosis of the infection as well as for bacterial genotyping [2].

Methodology

This study was conducted in a tertiary teaching hospital in Kelantan State, located in the northeastern coast of Peninsular Malaysia. Orthopedic ward entries in the Hospital Universiti Sains Malaysia database from 2008 to 2014 were retrospectively reviewed and analyzed for demographic data, clinical characteristics, management complications, and clinical outcomes. All patients diagnosed with melioidosis associated with bone, joint, or soft tissue, either primary or secondary to disseminated B. pseudomallei infection that originated from other organs, were included. All cases were confirmed from cultures of clinical specimens. Antibacterial treatment for acute-phase melioidosis used in the hospital during the study period was intravenous ceftazidime (40 mg/kg every 8 hours) or imipenem (20 mg/kg every 8 hours). For osteomyelitis and deep-seated soft-tissue abscesses, prolonged intravenous therapy was given, and the duration of treatment was decided based on clinical response and blood parameter of erythrocyte sedimentation rate

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(ESR) and C-reactive protein (CRP). During treatment, some cases required surgical interventions. For patients who presented with abscess formation, osteomyelitis, or septic arthritis, the operative management procedures included incision and drainage, corticotomy, curettage of the affected bone, arthrotomy, and washing of the affected joint. All patients with septic arthritis and deep-seated abscesses were managed surgically. The decision to operate on osteomyelitis was based on initial response to antibiotics and the presence of abscesses and chronic discharge.

Melioidosis cases were categorized based on the Infectious Disease Association of Thailand (IDAT) as transient septicemic melioidosis, bacteremic multifocal infection with septicemia, non-bacteremic localized infection, and bacteremic localized infection with septicemia [3].

Further investigations were done at the molecular level; archived *B. pseudomallei* previously isolated from clinical specimens were recalled from the stock culture laboratory and were reactivated and then subjected to polymerase chain reaction targeting type three secretion system (TTS1 PCR). In addition, multilocus sequence typing (MLST) that was performed as described previously [4]. The relatedness among isolates and the evolutionary divergence distance were estimated according on number of base differences per sequence (single-nucleotide polymorphisms [SNPs]) in the concatenated sequence of alleles at all loci using MEGA version 6 (Ibis Therapeutics, Los Angeles, USA).

Data entry and analysis were done using SPSS version 22 software (IBM, Armonk, Illinois, USA). Chi-squared or Fisher's exact tests were used to assess categorical variables; a p value of ≤ 0.05 was considered significant. Risk ratios and 95% confidence intervals were then calculated. Ethical approval was obtained by the Universiti Sains Malaysia research ethics committee (USM/PPP/JEPeM(235.4.(2.5))) (USM/PPP/JEPeM (235.4.(2.5))).

Results

From 20 cases collected, 19 patients were confirmed to have osteoarticular and/or soft-tissue melioidosis and were treated in the hospital from January 2008 to December 2014. Another case with soft-tissue infection was identified as *Burkholderia thailandensis* via genotyping and presented with localized infection. Eleven (55%) patients came from the same state (Kelantan), seven cases (35%) were referred from another state, Terengganu; the remaining two cases were from Selangor and Kedah.

The mean age was 43.47, with a range of 10 to 65 years. Four patients were young adults. The majority of patients were males (80%) (n = 16). Patients' gender was not associated with a higher risk for soft tissue than bone/joint melioidosis (p = 0.530 versus p = 0.582; risk ratio = 0.38 [95% CI 0.02-5.2]).

Sixteen (84%) patients had underlying diabetes; two of them were newly diagnosed as diabetes mellitus types I and II during the admission, and two diabetic patients had chronic renal disease; one patient who was an intravenous drug user had underlying HIV infection, and the remaining two patients had no identified underlying disease. In this study, there was no statistical difference between bacteremic and non-bacteremic melioidosis in relation to diabetes mellitus (83.3% versus 84.6%; risk ratio = 1.1 [95% CI 0.08–15.1]; p = 1.000).

All admitted patients presented with various complaints, including fever, chills, shortness of breath, and limited range of motion of the affected limbs with associated swelling, purulent discharge, or abscess formation. Almost half of the patients (n=10) presented with mild to severe sepsis, from which seven cases were complicated with septic shock requiring intensive care unit (ICU) care.

Distinct melioidosis types were seen in this report: bacteremic multifocal melioidosis (n = 9), in which B. pseudomallei was isolated from blood with the evidence of multiple organ dissemination; bacteremic localized melioidosis (n = 4), in which bacteria was isolated from blood specimens with the evidence of involvement; and single-organ non-bacteremic localized melioidosis (n = 6), in which bacteria was not isolated from blood but there was evidence of singleorgan involvement. Soft-tissue involvement was seen in 14 patients and was either deep muscle abscess (n = 6), skin cellulitis (n = 5), or both abscess and cellulitis (n = 5) 3). Septic arthritis and osteomylitis were seen in six patients. The twentieth case was a 42-year-old male with B. thailandensis infection manifested with nonbacteremic localized skin involvement who presented with ankle swelling, skin cellulitis, and a foot abscess. No predisposing factor was identified in that patient.

Emergency operative procedure and immediate acute-phase antibiotic administration for melioidosis patients were performed promptly. Six patients with soft-tissue abscesses and infected wounds underwent surgical drainage or wound debridement. Bone debridement and curettage were done in four cases with osteomyelitis, while arthrotomy and washout was conducted in four cases with septic arthritis. Six

patients were treated non-operatively with an acutephase antibiotic regimen.

Death due to melioidosis was documented in seven patients as a result of overwhelming septicemic shock, of which four patients were treated non-operatively. Death was a significant outcome in patients with bacteremia (p = 0.044). On the other hand, patients who were treated operatively (74%) were improved on short-term follow up for at least six months. One patient had relapsed. Three cases with sepsis were successfully treated after passing the acute-phase treatment. Two patients with mild infection and treated with antibiotic alone recovered well.

Out of 20 bacterial isolates, 19 were successfully amplified by TTS1 PCR. The remaining bacterial isolate that failed to amplify by TTS1 PCR was identified as *Burkholderia thailandensis* by MLST and assigned to genotype ST77. MLST revealed massive genotypic heterogeneity; out of 19 isolates, 14 different genotypes were identified and were genetically related to each other and to genotypes found in the Southeast Asia region. There was no evidence of strain differential virulence and tropism toward skin, muscle

bone or joint. In addition, there was no association between disease presentation and the clustering of isolates on the minimum evolution tree obtained with the concatenated sequences (Figure 1).

Discussion

B. pseudomallei infection was first described in 1912 by Whitmore and Krishnaswami in patients with fatal pneumonia. Melioidosis is a collection of disease states with variable clinical picture ranges, from benign tissue infections to rapidly fulminant and fatal septicemia. Melioidosis might be acute, chronic, latent, localized, and disseminated infection, and one form of the disease may progress to another [5]. Non-septicemic melioidosis could be presented with mild, subclinical, and localized infections with an overall mortality of 5%–20%, which increases in cases of acute melioidosis to 30%–47% [6]. Pneumonia and sepsis are the most severe clinical manifestations of melioidosis [3].

Patients with underlying illnesses including type 2 diabetes mellitus and immunocompromisation complicated with septicemic melioidosis can develop septic shock and abscess formation. The majority of

Age/Sex Melioidosis type Involved organs Location Risks Outcome Genotype ST54 20/Male Multifocal Lung,muscle,bone,joint Flbov **DM-2 Death ST54 DM-2,CKD** 50/Male Multifocal Kidney, liver, brain, muscle Thigh Death ST54 57/Female Bacteremiclocalized Skin, muscle, joint Buttok,knee,leg DM-2 Cured ST271 57/Male Multifocal Lung.joint DM-2 Death Knee ST306 51/Female NonBacteremiclocalized Slin, joint Foot,ankle DM-2 Relapse ST306 49/Male NonBacteremiclocalized Calf Muscle DM-2 Cured ST306 39/Male Bacteremiclocalized Skin, muscle Neck,hand DM-2 Death ST306 49/Male Multifocal Liver,spleen,joint Knee DM-2 Cured ST50 51/Male NonBacteremiclocalized Joint Cured ST46 56/Male Multifocal Skin,lung Forearm DM-2 Cured ST371 61/Male Bacteremiclocalized Bone Ankle,foot DM-2,CKD Death ST376 36/Male NonBacteremiclocalized Skin Neck HIV Cured ST1317 44/Female Multifocal Lung,skin,brain Forearm DM-2 Death ST1359 38/Male Bacteremiclocalized Skin Leg DM-2 Death ST289 Multifocal 65/Male Skin, joint, spleen Hand,ankle DM-2 Cured ST1319 10/Male Multifocal Muscle, liver, brain Thigh Nil Cured ST507 15/Male Multifocal Skin, muscle, liver Nil Forearm, calf Cured ST1323 17/Male Muscle DM-1 Cured NonBacteremiclocalized Thigh ST84 61/Male NonBacteremiclocalized Bone, joint Proximal tabia DM-2 Cured

Figure 1. Minimum evolution tree obtained with the concatenated sequences of B. pseudomallei genes combined with patient's history.

^{*}Refers to the primary organ of infection; **Diabetes mellitus; ***Death due to melioidosis. CKD: chronic kidney disease. The primary organ of infection is a simple of the primary organ o

patients with melioidosis are recognized as having underlying diseases: 76% in Malaysia, 88% in Australia, and 53% in Thailand [7]. However, the association between melioidosis and diabetes is strong and may increase the risk of the infection by up to 100-fold [8].

Bone and joint (osteoarticular) melioidosis can mimic acute or chronic infection or rheumatic arthritis, and may also resemble neoplasm, sarcoidosis, tuberculosis, and other granulomatous disease. In addition, bone and joint melioidosis could be difficult to differentiate from infections caused by other pathogens, except that the systemic features of the illness might be more prominent in melioidosis [9].

Several prospective studies had documented melioidosis of bone, joint, and soft tissue in endemic countries and reported various frequencies of bone/soft-tissue melioidosis episodes and different mortality rates that were directly proportionate to disease management (Table 1).

Melioidosis was not restricted to a certain age group; in this study, it was mainly reported in middle-aged adults and the elderly more than in children and young adults. Pediatric melioidosis is uncommon in endemic areas [10]; no cases of pediatric melioidosis were reported here, which might be attributed to the lack of predisposing factors such as those in adults.

In this study, the majority of subjects were males, as males have a higher incidence for melioidosis due to their greater exposure to environmental soil risk factors [7]. The predomination of male gender among osteoarticular melioidosis was also reported in the literature about bone and soft-tissue melioidosis [1,10]. Another risk factor reported in this study was diabetes mellitus, which has been reported to be the superior predisposing factor for melioidosis worldwide. Nonetheless, neither gender nor diabetes mellitus were more associated with one disease stage over another;

moreover, diabetes mellitus was the major predisposing factor for both bacteremic and non-bacteremic osteoarticular melioidosis, with no significant difference between the types of infection. The same finding was noticed for the effect of the gender risk factor on the site of infection, whether it was soft tissue or bone/joint. This supports the finding that male gender and diabetes mellitus have a predominant risk for all melioidosis types and stages and are not exclusive to a certain type.

Septic arthritis and osteomyelitis due to *B. pseudomallei* are rare but still considered well-recognized presentations of melioidosis [9]. In our study, only nine cases of osteoarticular involvement were reported over a seven-year review period, which concurs with other surveys [11,12]. The reason behind the low-rate involvement of osteoarticular tissue in melioidosis might be attributed to the low blood supply to these locations in comparing with other organs and, in parallel, the fact that the majority of melioidosis cases are bacteremic and affect mostly organs rich with blood supply such as liver, spleen, lungs, and brain.

Involvement of bones or joints in melioidosis usually occurs following the dissemination of B. pseudomallei from infection elsewhere in the body via different routes of spreading. However, septic arthritis and osteomyelitis can be the primary manifestations of melioidosis [10]. In this study, bone and joint infection due to secondary melioidosis were reported in six patients, occurring as a consequence of bacteremic multifocal or localized disease, whereas in three patients, bone and/or joint were the primary sites of melioidosis. Hassan et al. [13] reported seven patients with primary melioidotic osteoarticular illness, while only one case of hip-joint involvement secondary to lung melioidosis was reported by Deris et al. [12]. In another study performed in tropical Australia, the percentages of primary and secondary melioidotic

Table 1. Osteoarticular and/or soft tissue melioidosis in endemic countries.

	No. of patients ¹	Age	Study period	Osteoarticular /skin melioidosis ²	Country	Mortality ³	Reference
1	540	8m-91y	1989-2009	107	Australia	1.9%	[10]
2	27	15d-80y	2001-2005	5	Malaysia	20%	[11]
3	45	< 16y	1989-2013	38	Australia	NA	[12]
4	145	7y-83y	2005-2009	32	Malaysia	43%	[13]
5	781	40y-57y	1989-2012	50*	Australia	8%	[14]
6	536	1y-64y	1989-2009	41*	Australia	4.9%	[1]
7	25	18y-67y	2001-2007	20	India	NA	[15]
8	104#	41y-65y	1997-2000	25*	Thailand	NA	[16]
9	33	40y-65y	2006-2008	11*	Malaysia	18%	[17]
10	20	10y-65y	2008-2014	20	Malaysia	35%	This study

Total number of melioidosis patients diagnosed by culture and/or serology; Primary of secondary infection; Death rate in patients with primary or secondary osteoarticular/soft tissue melioidosis; Only septic arthritis and/or osteomyelitis; Patients with septic arthritis.

septic arthritis among 536 confirmed cases were 2.4% and 2.6%, respectively [1].

Pui and Tan [14] had retrospectively analyzed radiology images for 26 patients of musculoskeletal melioidosis and found that septic arthritis was more frequent than soft-tissue abscess and osteomyelitis in melioidotic patients. Skin and soft-tissue melioidosis was more involved as a primary or secondary infection in this report, as well as in other studies [11-15]. In the same study done by Pui and Tan [14], the knee was the most common infected location, followed by ankle, foot, shoulder, and hip; these results agree with our findings and those of other reports [10].

Death due to melioidosis in this study was more common and significant in patients with bacteremia due to the increased risk for developing severe sepsis and fatal septic shock ending in multi-organ failure. Similar results were reported previously and supported that fatal outcome is common in melioidosis, regardless the type of the infection and the involved organs [1,13].

Outcome of melioidosis management successful in 13 of 20 infected patients involved in this study (including the case of *B. thailandensis* infection). This result could be attributed to timely and appropriate acute-phase antibiotic therapy (ceftazidime and carbapenem) and early surgical intervention. However, among fatal cases, four patients who were treated nonoperatively presented with severe sepsis and septicemic shock that prevented surgical intervention. Therefore, failure to save those patients was not attributed to the lack of operative treatment; it might have been caused by delayed laboratory diagnosis with ineffective empirical treatment or delayed hospital admission. Moreover, some patients from endemic rural areas seek traditional medication first and/or wait for symptoms to clear. They only go to the hospital when disease symptoms worsen or fail to improve. It has been suggested that delayed diagnosis of more than two weeks is a risk factor for bone and joint involvement [16].

In the present study, several different *B. pseudomallei* genotypes were retrieved among typed strains in spite of a low number of cases. In addition, no genotypic predomination was recognized. These results were supported by the reported wide diversity of *B. pseudomallei* genotypes in a given geographical area, which reflects the dynamicity of bacterial spread via environmental routes as well as human transportation, a suggestion which is supported by the evidence of the presence of our genotypes in neighboring states and countries, such as genotypes ST84, ST289, ST376, ST371, ST46, ST50, ST306, ST271, and ST54.

Bacterial genotype heterogeneity was also reported in other studies that applied different genotyping methods in different countries [17-19]. On the other hand, *B. thailandensis*, known as environmental *Burkholderia*, is frequently isolated from soil and water, and is generally considered avirulent. Disease due to *B. thailandensis* is extremely rare; few cases have been reported [20,21]. There was no evidence of associations between *B. pseudomallei* genotypes and clinical presentations of osteoarticular melioidosis on the evolutionary tree, which is in agreement with previous reports [22,23].

Conclusions

The potential for primary melioidotic osteomyelitis and septic arthritis still exists, and an appropriate degree of suspicion is warranted for an accurate diagnosis and curative treatment. Diabetes mellitus remains the leading predisposing factors for melioidosis and its consecutive events of secondary organ involvement. So far, risk for genetic diversity of *B. pseudomallei* was not reported for melioidosis of any organ involvement. Early laboratory and clinical diagnosis of melioidosis can prevent further organ damage and decrease morbidity and mortality rates. In addition, acute-phase anti-melioidosis treatment followed by surgical intervention and repeated joint washouts and extensive debridement of infected bone improves the outcome of management and relapse prevention.

Acknowledgements

We would like also to thank Dr. Bader Tayyar for statistical guidance and Ali Abusafia for finalizing and proofreading the manuscript. We would also thank Azlan Abdullah and Nurleem Mursheed and hospital medical records unit in the USM for their help in the procurement of patients files and isolates. This project was funded by Malaysian Ministry of Education Exploratory Research Grant Scheme (ERGS) grant, no. 203/PPSP/6730024 awarded to Azian Harun.

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Conflict of interests: No conflict of interests is declared.