THE GEOGRAPHIC DISTRIBUTION OF \textit{Burkholderia pseudomallei} IN SELECTED MALAYSIAN LOCALES AND ITS EFFECT ON HUMAN EXPOSURE IN CHANGING LAND USE

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ABDUL KARIM RUSS BIN HASSAN

A thesis submitted in fulfillment of the requirement for the Degree of Doctor of Philosophy (Public Health Microbiology)

Faculty of Medicine and Health Sciences
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2012
Dedication

To my wife Normah, children Siti Amrah and Dzulkhaini.

To those who have suffered from melioidosis.
Acknowledgements

I wish to thank my supervisor Prof. Dr Hashami Bohari for his continuous support toward the completion of this thesis. I wish to record my appreciation to my co-supervisor Prof. Dr Timorthy John Jay Inglis from PathWest Laboratory Medicine and the University of Western Australia for his continuous support in guiding the research both in the field and laboratory work. I am indeed thankful to Prof. Dr Syed Hassan Al- Mashoor and Tan Sri Prof. Dr Taha Arif, Former Dean, Faculty of Medicine & Health Sciences, UNIMAS; Director of Medical and Health Services of Sarawak; Prof. Dr Mary Jane Cardosa, Former Director, Institute of Health and Community Medicine, UNIMAS; Prof Dr Puthucheary from Universiti Malaya Medical Centre, Sarawak Biodiversity Centre; Medical Laboratory Technologists of UNIMAS; Malaysian Diabetic Association, Sarawak Forestry Department, Erickson Air Crane Malaysia Sdn Bhd, Sarawak Bakun Hydro-Electric Project Management; staff of Microbiology Department, The PathWest Laboratory Medicine, Nedlands, Western Australia; Veterinary Research Institute, Sarawak General Hospital; Prof. Dr John Candlish and Assoc. Prof. Dr Gopalakrishnan for their advise, Universiti Kuala Lumpur Royal College of Medicine Perak for the use of research facilities. A special thanks to members of UNIMAS Research and Scientific Committee of Loagan Bunut Scientific Expedition, UNDP, and indigenous community leaders.

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Abstract

The aim of the study was to detect environmental sources of *Burkholderia pseudomallei* that cause melioidosis and to compare by molecular methods the environmental isolates with clinical isolates from endemic areas of Sarawak. The study was undertaken in view of reported cases of melioidosis from various government hospitals in Sarawak. Environmental isolation of *B. pseudomallei* and seroepidemiological survey for antibodies to *B. pseudomallei* was conducted in various indigenous communities in the periphery and within Loagan Bunut National Park, logging workers of Kakus in Bintulu, Bakun hydroelectric project workers, pottery workers in Kuching, commercial paddy workers in Gedong Paddy Estate and other people at risks in Sarawak. Soil and water samples from various ecological areas were screened by culture methods using a tripticase soy broth (TSB), enrichment threonine-basal salts solution and Ashdowns’s media. The kits for the analytical profile index (bioMerieux) and/or Becton Dickinson nonfermentor identification system were used for biochemical tests for identification of the environmental and clinical isolates. Further molecular detection of the *Burkholderia pseudomallei* and near neighbor species were conducted by PCR and a molecular typing by pulsed-field gel electrophoresis (PFGE). The genomic DNA of environmental and clinical isolates of *B. pseudomallei* were digested with restriction enzyme *Xba* 1. The patterns generated by PFGE were analysed for similarity, differences and the possible link between the environmental and clinical isolates of *B. pseudomallei* in relation to melioidosis cases in Sarawak. There was recovery of two *B. pseudomallei* from the logging areas of Kakus in Bintulu, one from Bakun in Belaga, and seven from Gedong Paddy Estate in Samunjan confirmed by PCR. The
study revealed heterogenous genomic variations in different environmental and clinical isolates of *B. pseudomallei* in Sarawak. PFGE demonstrated that two environmental isolates recovered from Kakus logging areas in Bintulu were indistinguishable from one clinical isolate from a patient admitted to Sibu Hospital and between clinical isolates from melioidosis cases in various hospitals were also evident. The data demonstrated a link between two environmental isolates in Bintulu and one clinical case in Sibu hospital and a relationship between the clinical isolates from various geographical locations. While in the occupational groups in Sarawak, ten of 31 (32.0%) of the pottery workers, seven of 47 (14.9%) of Bakun hydroelectric project workers and four of 17 (23.5%) logging workers were positive for *B. pseudomallei* exposure. However, all the 13 workers in commercial paddy cultivation in Gedong Paddy Estate, Semunjan were negative for *B. pseudomallei* exposure. Melioidosis remains an important occupational health hazard among the pottery, logging and hydroelectric project workers. In another study, an environmental soil survey was conducted in the peripheries of Loagan Bunut National Park, Sarawak. Soil samples from various ecological sites were screened by culture methods utilizing tripticase soy broth (TSB), and improved method of isolation in *Burkholderia pseudomallei* selective agar (BPSA). The reaction profile kits of the analytical profile index (API GN 32) for bacterial identification system (bioMerieux) was used for biochemical and monoclonal antibodies tests on the soil isolates. The identification of *B. pseudomallei* was further clarified with the use of PCR and confirmed to be *B. pseudomallei* in those location. Further risk monitoring surveillance was conducted on 142 single blood samples among the Iban, Berawan and Penan living in the periphery of Loagan Bunut National Park, northern Sarawak by the use of Indirect Haemagglutination Test (IHAT). The results showed high rates of exposure among
the Iban (20%), Penan (14.3%) and lowest among the Berawan (10%) communities. The difference in exposure rate among the ethnic groups were attributed to their agricultural practices and alteration to the environment. There were high isolation of *B. pseudomallei* in a secondary school field in Kuching, In northern peninsular Malaysia, the exposure to *Burkholderia pseudomallei* among the Orang Asli population living near the forest fringes in the district of Kuala Kangsar and Grik in Perak, those engaged in agricultural activities, army personnel and other occupational groups were determined. The total of 193 serum samples were screened of which 29% were positive. The results showed high exposures among the aboriginal Orang Asli communities, army personnel involved in field duties and those participating in outdoor activities. Environmental isolation of *B. pseudomallei* in various locations in paddy cultivation, school fields and in flood affected areas in Malaysia suggested the potential risk of melioidosis among those people. It is evident that melioidosis will be an important occupational health hazard among those rural communities engaged in agricultural activities and precautionary measures need to be taken amongst those involved in outdoor activities. The soccer fields need to have proper drainage and full grass cover. Students have to use proper attire such as wearing of adequate clothing to protect from contact with soil dan wearing proper boots during the soccer events.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>i</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>ii</td>
</tr>
<tr>
<td>Abstract</td>
<td>iii-v</td>
</tr>
<tr>
<td>Abstrak</td>
<td>vi-vii</td>
</tr>
<tr>
<td>Lists of Tables</td>
<td>xiii</td>
</tr>
<tr>
<td>Lists of Figures</td>
<td>xiv</td>
</tr>
<tr>
<td>Lists of Plates</td>
<td>xv</td>
</tr>
<tr>
<td>Lists of Photograph</td>
<td>xvi</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>xvii</td>
</tr>
</tbody>
</table>

## CHAPTER 1: GENERAL INTRODUCTION

1.0 Introduction 1

1.1 Aim and objective of the thesis 3

1.1.1 Aim 3

1.1.2 Objective 3

## CHAPTER 2: LITERATURE REVIEW: BURKHOLDERIA PSEUDOMALLEI AND MELIOIDOSIS

2.1 Early observation 5

2.2 Clinical features 7

2.3 Epidemiology 9

2.3.1 Current status outside Malaysia 9
CHAPTER 3: GENERAL MATERIALS AND METHODS

3.1 Study sites

3.2 Environmental soil sampling

3.3 Bacterial isolation and identification

3.4 Polimerase Chain Reaction (PCR)

3.5 Molecular typing

3.6 Seroepidemiological survey

3.6.1 Indigenous communities and other risk groups
3.6.2 Loagan Bunut National Park 37
3.6.3 Diabetic Camp in Semantan, Sarawak 37
3.6.4 Serum samples 42
3.6.5 Indirect Hemagglutination Test (IHAT) 42
3.6.6 Immunofluorescent Antibody Test (IFAT) 43

CHAPTER 4: MELIOIDOSIS AND CHANGING LAND USE IN SARAWAK 44-60

4.1 Introduction 44
4.2 Materials and methods 46
4.2.1 Study sites 46
4.2.1.1 Bukit Lumut, Bintulu in Northern Sarawak 46
4.2.1.2 Kapit, Central Sarawak 46
4.2.1.3 Beras Paddy Estate in Gedong,, Samunjan 47

4.3 Pulsed-field gel electrophoresis 47
4.4 Blood samples collection 49
4.5 Results 49
4.5.1 Environmental Isolation of Burkholderia 49
4.5.2 Blood samples for Indirect Haemagglutination Tests (IHAT) 54
4.5.3 PFGE typing 54
4.6 Discussion 56
# Chapter 5: Environmental Isolation of *Burkholderia* Species and Seroepidemiology of Melioidosis Among the Indigenous Communities in the Periphery and Within the Loagan Bunut National Park, Sarawak

5.1 Introduction 61
5.2 Materials and methods 61
5.3 Results 65
5.5 Discussion 69

# Chapter 6: Environmental Isolation of *Burkholderia Pseudomallei* in a School in Sarawak

6.1 Introduction 73
6.2 Materials and methods 74
6.2.1 Study sites 74
6.2.2 Samples collection 75
6.2.3 Bacterial identification 75
6.2.4 Semi-nested Polymerase Chain Reaction (PCR) 76
6.2.5 Molecular typing 76
6.3 Results 77
6.4 Discussion 83
CHAPTER 7: ENVIRONMENTAL SURVEILLANCE OF BURCHOLDERIA PSEUDOMALLEI IN SELECTED LOCATIONS AND SEROEPIDEMIOLOGICAL SURVEILLANCE OF MELIOIDOSIS AMONG THE COMMUNITIES AND OCCUPATIONAL GROUPS IN MALAYSIA 86-106

7.1 Introduction 85
7.2 Materials and methods 87
7.2.1 Study sites 87
7.2.1.1 Perlis and Kedah 87
7.2.1.2 Perak state 88
7.2.1.3 Johore State 89
7.2.2 Soil sampling 89
7.2.3 PCR 90
7.3 Seroepidemiological study area 91
7.4 Results 94
7.4.1 Environmental survey 94
7.4.2 Seroepidemiological survey 94
7.5 Discussion 97

CHAPTER 8: GENERAL DISCUSSION 100-105

LIST OF PRESENTATION AND PUBLICATION 106-107

REFERENCES 108-130
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PCR detection of <em>Burkholderia pseudomallei</em> from soil and water samples from Bukit Lumut logging areas in Bintulu</td>
</tr>
<tr>
<td>2.</td>
<td>Environmental survey for bacterial identification of Burkholderia and related species in Kapit, Sarawak by using the BBL Crystal Identification System</td>
</tr>
<tr>
<td>3.</td>
<td>PCR detection of isolates from soil and water samples for Burkholderia in Gedong Paddy Estate in Samunjan, Sarawak</td>
</tr>
<tr>
<td>4.</td>
<td>Soil sample collection for detection of Burkholderia spp. in various localities of Loagan Bunut National Park, Sarawak</td>
</tr>
<tr>
<td>5.</td>
<td>Single blood samples for detection of antibodies to <em>B. pseudomallei</em> among the indigenous communities of Loagan Bunut National Park, Sarawak</td>
</tr>
<tr>
<td>6.</td>
<td>Presumptive phenotypic and confirmatory genotypic identification of soil samples for <em>B. pseudomallei</em> from soccer field and secondary school compound in Kuching, Sarawak</td>
</tr>
<tr>
<td>7.</td>
<td>Distribution of <em>B. pseudomallei</em> isolated from soil samples from secondary school compound, Kuching, Sarawak</td>
</tr>
<tr>
<td>8.</td>
<td>Environmental isolation of <em>B. pseudomallei</em> in West Malaysian locales</td>
</tr>
<tr>
<td>9.</td>
<td>Single blood samples for detection of antibodies to <em>B. pseudomallei</em> from IFA test among adults Orang Asli (Aboriginal communities), occupational groups, army personnel and civilian workers in Malaysia</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure

1. Results of pulse field gel electrophoresis (PFGE) of environmental *Burkholderia pseudomallei* numbers 31, 40, 31 and clinical isolates from East Malaysia.

2. PFGE of environmental soil isolations of *B. pseudomallei* numbers NEQ530*, TIA 830*, GR130*, GR360, NEQ2530* from secondary school in Kuching and clinical isolates from Sarawak and West Malaysia.
LIST OF PLATES

Plate

1. Maps of study sites in Sarawak
2. Map showing reported cases of melioidosis and soil sampling for *B. pseudomallei* in Sarawak
3. Map of Northern Sarawak showing the location of Loagan Bunut National Park, Northern Sarawak
4. Map showing soil and blood samples survey sites in Loagan Bunut National Park.
5. Map showing study sites in Ulu Kelantan in Northern Peninsular Malaysia.
6. Map of West Malaysia showing locations (blue flags) of soil samples collection for *B. pseudomallei* in various land use
7. Map of Sarawak showing locations (blue flags) of soil samples collection for *B. pseudomallei* in various land use
LIST OF PHOTOGRAPH

Photograph

1. Gedong Paddy Estate, Semunjan for soil sampling areas
2. Blood samples collection from the Iban community in Loagan Bunut National Park
3. Respondents for blood sample collection among the indigenous community of Sg Gelong, Loagan Bunut National Park
4. Blood samples collection from the indigenous community in Loagan Bunut National Park
5. Respondents in Diabetic Camp for blood samples collection
6. Innoculation of school soil sample into Tryptic Soy Broth (TSB) showing pellicles on the surface suggestive of *B. pseudomallei*
7. Environmental soil isolates from school soccer field on *Burkholderia pseudomallei* selective media (BPSA) showing wrinkles colonies after 72h incubation
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>API</td>
<td>Analytical Profile Index</td>
</tr>
<tr>
<td>BPSA</td>
<td><em>Burkholderia pseudomallei</em> Selective Media</td>
</tr>
<tr>
<td>DEIA</td>
<td>Dot Enzyme Immunoassay</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunoabsorbent Assay</td>
</tr>
<tr>
<td>GPS</td>
<td>Geographical Positioning System</td>
</tr>
<tr>
<td>IFA</td>
<td>Immunofluorescence Antibody Assay</td>
</tr>
<tr>
<td>IHA</td>
<td>Indirect Haemagglutination Assay</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>LB</td>
<td>Lucia Broth</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulsed-field Gel Electrophoresis</td>
</tr>
<tr>
<td>PGP</td>
<td>Phosphotidyl Glycerol Phosphatase</td>
</tr>
<tr>
<td>PHB</td>
<td>Poly β-Hydroxybutyrate</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction Fragment Length Polymorphism</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>ST</td>
<td>Sequence Typing</td>
</tr>
<tr>
<td>TSB</td>
<td>Trypticase Soy Broth</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra Violet</td>
</tr>
</tbody>
</table>
CHAPTER 1

GENERAL INTRODUCTION

1.0 Introduction

The review discusses an early report of melioidosis in Burma (now Myanmar) that was first described by Whitmore and Krishnaswamy in 1911 and subsequent report of infections in laboratory animals in the Federated Malay States (now West Malaysia) at the Institute for Medical Research, Kuala Lumpur (Whitemore & Krishnaswami, 1912 and Stanton & Fletcher, 1932). The review considers reports of clinical epidemiology of melioidosis that occurred after several decades in other parts of South East Asia after World War II and the worldwide distribution of melioidosis in Australia, Asia, Americas and other tropical countries. The clinical features and other factors contributing to the exposure among the different ethnic communities and occupational groups were discussed.

It also included an account of the characteristic features and morphology of Burkholderia pseudomallei grown in various culture media. They were early reports of the environmental survey in endemic areas for the distribution of B. pseudomallei based on several isolation methods and isolation of Burkholdria pseudomallei in clinical cases from different geographical region. This was followed by molecular typing methods of B. pseudomallei that involved DNA macrorestriction analysis of the various environmental and clinical isolates.
*B. pseudomallei* as a cause of melioidosis affect people living in endemic areas that includes those engaged in agricultural activities in various land utilized for commercial crops and subsistence agriculture. In Malaysia, paddy cultivation have been practiced for centuries as rice formed the staple food for the growing population. In commercial paddy cultivation, most of these people planted paddy by using machinery. The indigenous people of Sarawak planted paddy by using traditional methods practiced in dry and wet paddy cultivation. In dry paddy cultivation, prior to planting the areas were cleared by burning the paddy stumps after the harvest. They were possible contact to *B. pseudomallei* among the farmers during their agricultural activities.

In Sarawak, there is a necessity for construction of hydroelectric power to support the energy demand for the increasing population and the setting up of more industries in urban areas. Large areas of land were logged for the construction of dam in Bakun hydroelectric project. Massive earthworks would results in the disturbance of the soil that would affect the equilibrium of the microbial diversity as large areas of forested areas were cleared. The project involved thousands of construction workers, the use of heavy machineries and resettlement of the indigenous people from the construction sites. The Sarawak Health Department reported several cases of melioidosis with mortality among the workers.

The indigenous people of Loagan Bunut National Park in northern Sarawak live in the periphery and the vicinity of the park. They settled in long houses situated along the Tinjar river and near the lake. The river Tinjar formed an important route for transportation of timber and for movements of the people. Most of them are engaged in paddy cultivation and fishing.
Large forested areas in Sarawak have been logged with the construction of many timber camps and increased mobility of logging workers. Access timber roads were constructed in the inland areas resulting in exposure of ground surface and erosion during heavy rain that drained into the river system. High sedimentation occurred in the rivers and large amount of soil suspension flowed into the rivers during the rainy season. Helicopter logging were introduced to extract timber from the high terrain. The timber industries employed thousands of workers engaged in the logging activities. These workers were exposed to \textit{B. pseudomallei} and some of them developed melioidosis as reported by Sarawak Health Department among the workers of the logging companies. They were possible distribution of \textit{B. pseudomallei} in various areas of logging concession, hydroelectric project and agricultural land use.

1.1 Aim and Objectives of the Thesis

1.1.1 Aim

This study aims to determine the geographic distribution of \textit{Burkholderia pseudomallei} in Malaysia and its implications on the human population.

1.1.2 Objectives

i. To determine the geographic distribution of \textit{Burkholderia pseudomallei} in selected Malaysian locales in changing land use.

ii. To determine the likelihood of human exposure of \textit{Burkholderia pseudomallei} in changing land use in those locations.
iii. To identify specific occupational and other activities that lead to environmental *B. pseudomallei* exposures.

iv. To establish future priorities for melioidosis surveillance, prevention, control and health promotion.

The study was to recover *B. pseudomallei* and other related species during an environmental survey in various geographical areas, land use and other ecological locations that were less known to have been previously surveyed in Sarawak and West Malaysia. The environmental surveillance for soil samples collection was conducted in areas where there were reported cases of melioidosis mortality or morbidity and high exposure to *B. pseudomallei* among the population and occupational groups in Malaysia. The study included the molecular detection and determination of genotyping of *B. pseudomallei* isolates from the environmental and clinical isolation that would have the possible link with infection in specific localities.

In addition, a seroepidemiological surveillance for blood samples collection was conducted to detect antibodies to *B. pseudomallei* among the population residing in rural areas and occupational groups believed to have a higher risk of infection.

The study would allowed further understanding of people likely at risk of contacting melioidosis in a given location and to formulate preventive measures to minimize disease transmission. In addition, the determination of those people at risk will allow future primary preventive measures by the availability of a vaccine to those high risk groups of people.
CHAPTER 2

LITERATURE REVIEW: MELIOIDOSIS AND BURKHOLDERIA PSEUDOMALLEI

2.1 Early Observations

Meliodosis is a potentially fatal disease of man and animals caused by a saprophytic bacterium, *Burkholderia pseudomallei* which is present in soil and surface waters, mainly in tropical countries. The bacterium was first discovered to be the causative agent of melioidosis, based on Koch’s postulate, by inoculation of the culture intraperitoneally into guinea-pigs by pathologist Alfred Whitmore and bacteriologist C. S. Krishnaswami in 1911. The first human infection was discovered in Rangoon, Burma (now Myanmar) during an autopsy of a 40-year-old morphine addict. Cultures of the lobar consolidation in his lung grew motile bacilli (Whitmore & Krishnaswami, 1912). However in 1917, Krishnaswami continued to report up to 10% cases of melioidosis in all autopsy deaths in Rangoon. Since 1945, a limited number of cases were reported among travelers to Myanmar (Hsueh *et al.* 2001 and White, 2003).

In the Federated Malay States (now peninsular Malaysia) the disease was first recognised in 1913 by Stanton and Fletcher. The term melioidosis was coined and derived from Greek word melis meaning “a distemper of donkey (asses)” and eidos, “resemblance” after studying an outbreak in laboratory animals, especially guinea-pigs that were infected with *Pseudomonas pseudomallei* at the Institute for Medical Research, Kuala Lumpur (Stanton & Fletcher, 1932). Human melioidosis was