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Environmental Surveillance for Potential Human Exposure to *Burkholderia Pseudomallei* Causing Melioidosis in Changing Land Use in East Malaysia

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Abstract

Melioidosis is a potentially fatal disease caused by saprophytic bacteria, *B. pseudomallei* that are present in soil environment in tropical countries especially in South East Asia. The study was to determine the distribution of *B. pseudomallei* in changing land use in East Malaysia and the exposure among the communities. The soil samples were taken from school compound; Gedong Paddy Estate, villages and logging areas of Kakus in Bintulu were screened by culture methods and confirmed by Polymerase Chain Reaction (PCR). Blood sample from various occupational groups were taken for seroepidemiological exposure to *B.pseudomallei* by using Indirect Hemagglutination Antibody Test (IHAT). There were isolation of 23 sites for *B. pseudomallei* from the quadrants in football field and other playing ground in the one school compound in Kuching, Sarawak, East Malaysia; two sites in Lumut logging areas and seven sites in commercial paddy cultivation in Gedong Paddy Estate in East Malaysia. In other occupational groups, Bakun hydro-electric project workers (8.75%) and logging workers (23.53%) were positive for *B. pseudomallei*.

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Awareness of the disease among the health professional and those high risk communities need to be address to prevent further contact with the bacterium. Protective measures during outdoor activities and those engaged in agricultural activities need to be formulated. Environmental protection for sustainability to prevent further deterioration and proliferation of the pathogens need to be prevented.

Keywords: Melioidosis; seroepidemiology; *Burkholderia pseudomallei*; Indirect Hemagglutination Test (IHA)

1. Introduction

Melioidosis is endemic in Southeast Asia and Northern Australia. An early report of environmental isolation of *Burkholderia pseudomallei* in soil and surface water were conducted around Saigon, Vietnam with a positive recovery of 3.3% [5]. They were high incidence of melioidosis amongst USA helicopter crews of soldiers during the Vietnam War [22]. A cluster of acute melioidosis cases were reported among the remote coastal communities in tropical Western Australia, resulting in the recovery of *Burkholderia pseudomallei* isolates from the soil in the community [10].

In West Malaysia, an early survey showed high percentages of isolates of *B. pseudomallei* in soil of cleared fields and wet rice fields [6,19]. In Sabah, report of 2.9% of soil and water samples was positive for *B. pseudomallei* in some of the coastal areas [19]. It is believed that the disturbance of the soil by various human activities may have an influence on the bacterial density. Melioidosis among workers involved in helicopter in timber logging and other ground logging activities were reported by the logging company and the Sarawak Health Department. Environmental surveys for *Burkholderia* in other parts of East Malaysia, particularly in Sarawak, are scarce. This study aimed to establish the endemicity of melioidosis, and to map the occurrence and isolation of *B. pseudomallei* in Sarawak.

In addition, the study was to isolate *Burkhoderia pseudomallei* and its distribution from the school compound where the students performed their sport activities. They were reported cases of melioidosis among secondary school students by the health department and reported cases of melioidosis from Kuching General Hospital, Sarawak with spectrum of organ involvement. Molecular typing by PFGE on environmental and clinical isolates was conducted to ascertain whether they were linked with melioidosis patients admitted to hospitals in Sarawak**.**

2. Material and Methods

The study were conducted in five areas of Sarawak comprising of timber logging areas Bukit Lumut, Bintulu in northern Sarawak; villages of indigenous communities of Kapit, Hydroelectric Project in Belaga in Central Sarawak, commercial paddy estate in Gedong, Samunjan in southern Sarawak and school compound in Sarawak. A total of 62 soil and 33 water samples were taken from Kakus, Bukit Lumut logging areas.

Nineteen soil samples from Malay villages of Kampung Muhibah and another twenty samples from Kampung Melayu Baru situated near Kapit town and five water samples were collected from the nearby bank of Rejang River. Other study sites were located in Iban villages of Lampung Balleh where twenty soil samples were obtained from their small holdings. Fourty-three soil samples were obtained from Nyalambung. Nine soil

samples were taken from the sites of Bakun hydroroelectric project that was under construction. Thirty-six soil samples and ten water samples were taken from two plots of commercial paddy cultivation operated by Gedong Paddy Estate in Gedong, Semunjan in southern Sarawak.

Approximately three cm^3 soil samples were taken from the surface and at 30 cm depth by using an augur and collected into sterile universal bottles containing 2 ml of distilled water. Soil samples from the localities were also inoculated into Gallimand and Dodin broth media and Ashdown's broth (1.2.3). Subsequently sub-cultured in Ashdown's agar. Presumptive identification of B. pseudomallei and related species were subjected to 21 biochemical tests by using API 20NE (Analytical Procedure Index Bio-Mérieux) and BBL Crystal Identification System. The procedures were carried out in accordance with the manufacturers' instruction [5].

A semi nested polymerase chain reaction (PCR) was conducted with the use of a pair of primers for further confirmation of *B. pseudomallei* from the environment and clinical samples to improve the detection method. First round primers consist of bp1 (5'-CGATGATCGTTGGCGCTT) and bp4 (5'-CGTTGTGCCGTATTCCAAT), followed by semi-nested second round primers bp1 and bp3 (5'-ATTAGAGTCGTCGAACAAT) [12].

Both the clinical and environmental isolates were characterized by pulsed-field gel electrophoresis (PFGE) to determine the link between the environmental isolates with the clinical isolates from patients admitted in hospitals. The prepared DNA was digested with restriction enzyme, *Xba 1* and subjected to pulsed-field gel electrophoresis in accordance with the protocol adopted from PathWest [11]. A gel was scanned by using Quality One software to ascertain a dendogram and for evidence of closely related pattern of isolates when a group of cases of melioidosis occured in the community.

Single blood samples were taken from workers in the logging camps in Kakus,and among the workers of hydroelectric projects in Bakun in central Sarawak and commercial paddy workers of Beras Paddy Estate, Gedong, South Sarawak. The serum samples were stored at -20^0C until determination of antibodies to *B. pseudomallei* by the use of Indirect Haemagglutination Test (IHA).

3. Results

Blood Sample for Indirect Haemagglutination (IHAT)

Four of the 17 (23.53%) logging workers were positive for *B. pseudomallei* antibodies with a cut-off titre of 1:40. Seven of the 47 hydroelectric project workers (14.89%) were positive for *B. pseudomallei* antibodies. All 13 workers from Gedong Paddy Estate were negative for antibodies to *B.pseudomallei*.

PFGE Typing

The study revealed heterogenous genomic DNA variations of different *B. pseudomallei* from environmental and clinical isolates from patients admitted in Sarawak hospitals.

Table 1: Environmental isolation of Burkholderia and related species by using BBL Crystal Identification System from Kapit, Sarawak by using BBL Crystal Identification System

No.	Code No	Location	BBL Crystal
1	KMS 4	Kg Melayu Baru/River Rejang Bank	***B. cepacia***
2	KMS 5 30	Kg Melayu Baru/River Rejang Bank	***B. cepacia***
3	S 5	Tributaries of Balleh, Sg Sut	***B.cepacia***
4	SW 3	Tributaries of Balleh, Sg Sut	***B. cepacia***
5	Sut S 30	Tributaries of Balleh, Sg Sut	***B. cepacia***
6	BS 1	Kg Beletek Baru/Kg Muhibah	***B. cepacia***
7	BS 2	Kg Beletek Baru/Kg Muhibah	***B. cepacia***
8	BS 3	Kg Beletek Baru/Kg Muhibah	***B. cepacia***
9	BS 4	Kg Beletek Baru/Kg Muhibah	***B. cepacia***
10	BS 7	Kg Beletek Baru/Kg Muhibah	***B. cepacia***
11	BS 7*	Kg Beletek Baru/Kg Muhibah	***B. cepacia***
12	BS 8	Kg Beletek Baru/Kg Muhibah	***B. cepacia***
13	BS 9 30	Kg Beletek Baru/Kg Muhibah	***B. cepacia***
14	BS 10	Kg Beletek Baru/Kg Muhibah	***B. cepacia***
15	BS 15	Kg Beletek Baru/Kg Muhibah	***B. cepacia***
16	NS 9	Rumah Nylambung/Orchid/Ladang	***B. cepacia***
17	NS 23	Rumah Nylambung/Orchid/Ladang	***B. cepacia***
18	NS 30	Rumah Nylambung/Orchid/Ladang	***B cepacia***
19	FP 1	Rumah Nylambung/Fish pond	***B. cepacia***

Table 2: PCR detection of *Burkholderia* from soil and water samples from Bukit Lumut logging areas in Bintulu, Kg Muhibah and Kg Melayu in Kapit, Serawak

No.	Samples	Location	Oxidase test	Gram Stains Structures	PCR Identification
1	BLW 11	Bukit lumut	+	Gram negative rod	*B. cepacia*
2	KBS 23	Bukit Lumut	+	Gram negative rod	*B. pseudomallei*
3	KB 34	Bukit Lumut	+	Gram negative rod	*B. cepacia*
4	KBS 39	Bukit Lumut	+	Gram negative rod	*B. pseudomallei*
5	KB 48	Bukit Lumut	+	Gram negative rod	*B. cepacia*
6	KBS 58	Bukit Lumut	+	Gram negative rod	*B. cepacia*
7	PF 1 13/7	Nyalambung	+	Gram negative rod	*B. multivoran*
8	BS 3 11/2	Kg Muhibah	+	Gram negative rod	*B. vietnamensis*
9	KMS 6	Kg Melayu	+	Gram negative rod	*B. multivoran*

PFGE of the strains revealed two environmental isolates recovered from a former logging camp and the proposed new camp in the Kakus logging areas of Bintulu were indistinguishable. These environmental isolates were also indistinguishable from the clinical isolate of a patient admitted to Sibu Hospital. Close correlation among the patterns of strains of clinical isolates from melioidosis cases of various hospitals were also evident. The data linked the environmental isolates in Bintulu, the clinical case in Sibu hospital and the clinical isolates from various geographical locations (Figure1).

Table 3: PCR detection of soil and water samples for Burkholderia in Gedong Paddy Estate, Semunjan, Sarawak

No.	Samples	Location	Oxidase test	Gram Stains structures	Colony morphology	PCR Identification
1	GS 6	Gedong	positive	Gram negative rod	creamy & circular	***B. multivoran***
2	G11 30	Gedong	positive	Gram negative rod	Creamy& circular	***B.cepacia***
3	GS 12	Gedong	positive	Gram negative rod	Creamy & circular	***B. multivoran***
4	G15 30	Gedong	positive	Gram negative rod	Creamy & circular	***B. multivoran***
5	G16 30	Gedong	Positive	Gram negative rod	Metallic & circular	***B.pseudomallei***
6	G18 30	Gedong	Positive	Gram negative rod	Creamy & circular	***B. multivoran***
7	PGS 1	Gedong	Positive	Gram negative rod	Creamy & circular	***B. pseudomallei***
8	PG 2 30	Gedong	Positive	Gram negative rod	Creamy & circular	***B. pseudomallei***
9	PGS 7	Gedong	Positive	Gram negative bacillus	Circular & creamy	***B. pseudomallei***
10	PG 7 30	Gedong	Positive	Gram negative rod	Creamy & circular	***B. multivoran***
11	GW 1	Gedong	Positve	Gram negative rod	Creamy & circular	***B. pseudomallei***
12	GW 9	Gedong	Positive	Gram negative rod	Creamy & circular	***B. pseudomallei***
13	GW 10	Gedong	Positive	Gram negative bacillus	Creamy & circular	***B. pseudomallei***

Table 4: Distribution of *B. pseudomallei* isolated from soil samples from secondary school compound, Kuching, Sarawak.

Location	Quadrant	Total samples cultured	***B. pseudomallei*** Isolated (%)
	Southeast Quadrant (SEQ)	26	3 (11.54)
Soccer field	Northeast Quadrant (NEQ)	23	11 (47.82)
Playground area	Northwest Quadrant (TIA)	22	3 (13.63)
Telematch area	Telematch Quadrant (TIA)	24	6 (25.00)
Total		95	23 (24.2)

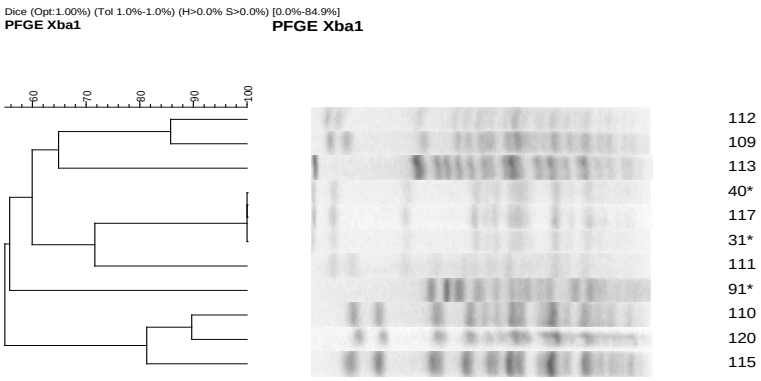

Figure 1: Results of pulse-field gel electrophoresis of environmental *Burkholderia pseudomallei* numbers 31*, 40* and 91* and clinical isolates from East Malaysian isolates.

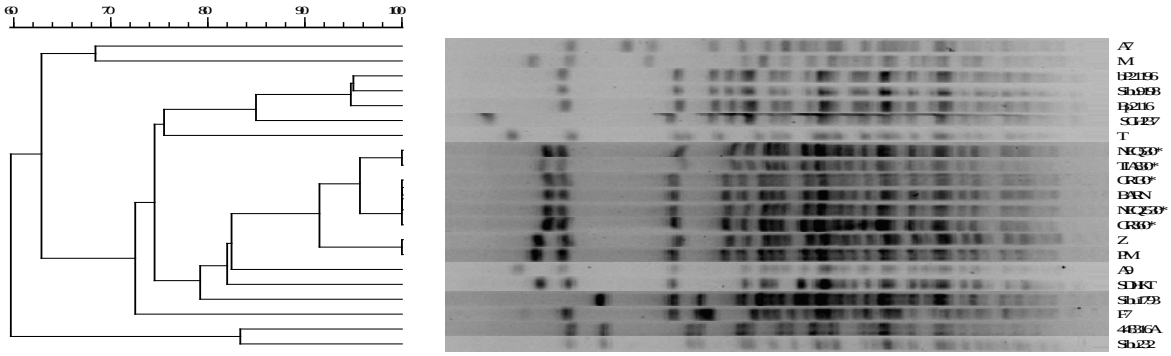

Figure 2: PFGE of environmental soil isolations of *B. pseudomallei* numbers NEQ530*,TIA 830*, GR130*, GR360, NEQ2530* from secondary school in Kuching and clinical isolates from Sarawak and peninsular Malaysia.

4. Discussion

The detection of *Burkholderia pseudomallei* in the Bukit Lumut logging areas of Bintulu suggests that it is the source of melioidosis in the area and a potential occupational risk to the workers. Aerosols from the soil particles containing the bacterium are dispersed into the air during earth works and the dust particles containing the *B. pseudomallei* duringmovement of the helicopter logging in the area may contribute to the transmission of the disease to humans by inhalation of the contaminated dust particles. This was evidence in another study on the prevalence of melioidosis among helicopter pilots and crews during the Vietnam War due to inhalation of contaminated dust [9,17].

The long hours of helicopter logging plying around the logging areas would help to disperse the surface soils into the air allowing the bacterium to remain in the suspended particles. Helicopter logging operation in Kakus

was within two kilometers from the logging camps. The cut timber were transported by helicopter to the landing area ready to be loaded onto a lorry near the base camp. At the landing area, the helicopter took a resting cycle every hour for 30 minutes without stopping the rotor while the engineer did the inspection. This would cause further disturbance on the surface soil due to strong turbulence generated from the rotor. Four of the seventeen logging workers were exposed to *B. pseudomallei* during the study. They included the loadmaster who was at the logging site during the operation. However, the potential risk of causing the disease would depend on the concentration and the virulence of the bacterium in the environment, host immune status and the underlying disease in the host [8].

Figure 3: Map showing reported cases of melioidosis and soil samplings for Burkholderia in Sarawak

Reported cases of melioidosis among two logging workers and a logging camp manager who were admitted to Kapit, Sibu and Christchurch Hospital in New Zealand would support the investigation on the presence of the bacteria in the soil from the study areas. Increasing numbers of reported cases involving timber workers in the areas suggest the need for preventive measures, especially to avert incidence of trauma as a result of fractures and multiples injuries with open wounds. A probable route of transmission is through inoculation or inhalation from contaminated soil. It was reported that patients with melioidosis admitted 1-2 weeks after heavy monsoon

are more ill and more likely to die. These patients were almost twice as likely to have bacteriaemic pneumonia if the rainfall in the 14 days before admission was more than 125 mm [4]. As a precaution against infection among those with skin and soft tissues injuries, immediate wound debribement and prompt treatment with appropriate antibiotics have been advised.

The levelling and clearing of land during construction of logging camps and timber tracks would result in massive movement of soil that may lead to erosion of the top soil favorable for the proliferation of bacteria especially during the rainy season. This was evident in the isolation of *B. pseudomallei* in the vicinity of the disturbed soil in the areas. During rainy season, soil erosion from the logging areas containing *B. pseudomallei* may be washed into the rivers and flowed in its tributaries in the coastal areas of Rejang river basin toward the residential areas of Sibu and other lowland areas where they were cases of melioidosis in government hospitals as reported from the Sarawak Health Department. Excessive logging activities in the upper river and clearing of forest would contribute to further erosion during the rainy season. In other studies in southeast Asia, the northeast Thailand were endemic for melioidosis where the Mekong river system carried silt during the wet season to Laos, Cambodia and Vietnam [15,21]. There was high isolation of *B. pseudomallei* from the soil beyond the vicinity of Mekong river in southern Laos [16].

Low recovery of *B. pseudomallei* could be due to the prolonged dry season in Sarawak. In northeastern Thailand, the incidence of melioidosis coincided with the rainy seasons with possible movement of *B. pseudomallei* towards the surface of the soil. In Northern Territory, Australia, increasing number of cases coincided with rainy season in 33 cases of melioidosis with 12 deaths that were reported at Royal Darwin Hospital between November 1990 to June 1991 [14]. In the preliminary survey of primary forest of Pa Rabata in northern Sarawak, soil samples was not detected for *B. pseudomallei.* The study was consistent with very low isolation of *B. pseudomallei* in primary forest suggested that the undisturbed forest remains in the state of equilibrium [19].

The use of Ashdown's media, in this study, resulted in the isolation of predominantly *B. cepacia* from the environmental soil and surface water in Bukit Lumut in Bintulu and several settlements in Kapit. Most *B. cepacia* and *B. pseudomallei* strains shared similar resistance to gentamicin and colistin. Both species were able to grow at a temperature of 37^{o}C in Ashdown's media making growth discrimination difficult. Ashdown's media was used for isolation of *B. pseudomallei* with varying recovery rates in environmental surveys in northern Australia and Thailand [2, 23].

The failure to recover *B. pseudomallei* in the other study areas suggest further environmental surveillance for *B. pseudomallei* in more areas which had evidence of community exposure towards the organism as reported in seroepidemiological studies and reported cases of melioidosis by Sarawak Health Department.

The use of PFGE in the study for typing of *B. pseudomallei* by using *Xba* 1 was suitable for both environmental and clinical isolates. The studies have demonstrated that clinical cases of melioidosis admitted to Sibu Hospital could be linked to indistinguishable environmental isolates from Kakus logging camp. However, the other strains from clinical cases of melioidosis from hospital in Sarawak showed high degrees of heterogenicity.

The use of machinery by the workers in Gedong Paddy Estate minimised contact with the soil as no report of *B. pseudomallei* infection among them. They were using trackter during the planting season in the month There was an effect of fertilizers activators on the growth of organic nitrogen bacteria and actinomycetes growth in the soil [7]. There was significant increase in microbial counts especially those soil treated with triple super phosphate followed by urea plus triple super phosphate, cow manure and urea.

There was an increase in the count of *B. pseudomallei* in Northern Territory of Australia after treatment of the soil with organic fertilizer and those treated with Nitrogen, Potassium and Phosphate [13]. Further research that need to be done on the growth of *B. pseudomallei* in paddy cultivated areas in areas with the use of organic fertilizer as compared to chemical fertilizers.

The isolation of a cluster of *B. pseudomallei* in the school compound especially the soccer field, suggests a potential source of infection to students and others who were in contact with the bacteria in the soil. During the wet season, certain areas in the soccer field were poorly covered with grass and the area were prone to accumulation of water during rainy season especially in front of the goal mouths, the centre of intense activities occurs during the games. These areas on the soggy and wet ground were noted to yield more isolates of *B. pseudomallei*. There was a possibility of the players fell into the puddles and sustained skin abrasion leading to contact with the contaminated soil.

Molecular typing by PFGE demonstrated that the environmental isolates of *B. pseudomallei* showed clusters of indistinguishable strains of *B. pseudomallei* recovered from the school compound as seen in Figure 2. These clusters of soil isolates were indistinguishable from the clinical isolates from one patient who had died of melioidosis in Sarawak General Hospital, Kuching. He was a resident in Bau about 20 km away from the school.

Several measures need to be taken to prevent transmission of melioidosis from the environment that includes wearing of protective clothing and proper shoes or boots during sport and physical activities. Proper sport attire during physical activities would prevent injuries especially to the skin and minimise skin contact with the bacteria. Precaution during sport events and game is essential to prevent injuries that would result in transmission of infection through contact with the contaminated soil. Adequate wound decontamination techniques for skin injuries is essential to prevent disease transmission and by taking appropriate antibiotic treatment for possible infection.

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