Distribution of melioidosis cases and viable *Burkholderia pseudomallei* in soil: Evidence for emerging melioidosis in Taiwan

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Running title: Distribution of *B. pseudomallei* in Taiwan

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Abstract

A survey of *B. pseudomallei* soil prevalence in Taiwan is comparable to melioidosis endemic region elsewhere. Presence of identical genetic patterns among clinical and environmental isolates suggested the link between pathogens in contaminated soil and an emergence of indigenous melioidosis.
Melioidosis is a serious infection caused by *Burkholderia pseudomallei*, a soil-dwelling saprophyte mainly distributed in an area between the latitude 20°N and 20°S (1). Human infection usually occurs by inhalation or subcutaneous inoculation with contaminated materials (4). In Thailand, most patients with melioidosis are farmers who contract the bacteria from a heavy exposure to *B. pseudomallei* during their agricultural activities (2). Several reports have documented the association between disease incidence and the environmental prevalence of *B. pseudomallei* (2,10,11).

In Taiwan, melioidosis was first reported in 1984 when a traveler returned from the Philippines (6). From 1984 to 2000, only 20 cases of melioidosis in Taiwan were reported (5). These cases were categorized as being acquired during prior travels to endemic areas overseas rather than indigenously acquired because the confirmed cases were rare and the pathogens were never isolated from the environment. However, the number of melioidosis cases suddenly increased in Er-Ren River Basin at southern Taiwan after a typhoon followed by a flood in 2005 (8, 9). *B. pseudomallei* was isolated from cropped soil and the *B. pseudomallei* specific antibodies were significantly increased among residents after this incident (9). This raises the question as to the extent *B. pseudomallei* is found in soil (nature environment) of Taiwan.

Thus, the soil samples from the cropped fields were collected from October 2005 to March 2007. Each sampling site was 5 km or 10 km apart from the other throughout
Taiwan. The cropped fields were sampled by digging three separate holes. The digger was disinfected with 70% alcohol between soil collections. A total of 1053 soil specimens were collected. Approximately 100 g of each sample was obtained at a depth of 30-60 cm and placed (15 g) into 50 ml of Ashdown’s broth in a 250-ml flask. The samples were processed and enumerated for the typical dry, wrinkled, violet-to-purple colonies of *B. pseudomallei* (9). The clinical isolates (n=6) obtained from melioidotic patients who had never traveled overseas were also analyzed (3). Biochemical tests (API system; bioMérieux, France) and molecular diagnosis (presence of the specific amplicons for the 16S RNA [243 and 405 bp] and flagella genes [267 bp]) were used for confirmation of *B. pseudomallei* (9). Total DNA in soil was isolated and purified with soil genomic DNA extraction kits (GeneMark, Taiwan) and purification kits (IsoQuick; ORCA Research Inc. USA), respectively. If both amplicons of 16S RNA and flagella gene were amplified from soil total DNAs, it was concluded that *B. pseudomallei* was present in the soil sample. The genetic relatedness among the clinical and environmental isolates was determined by RAPD (randomly amplified polymorphic DNA)-PCR and PFGE (pulsed-field gel electrophoresis) analysis. The RAPD-PCR primer used was GEN2-60-09 (5′-CCTCATGACC-3′) and a standardized protocol was followed (7). The PFGE was performed in a CHEF-III DR system using *XbaI* and *SpeI*-digested high molecular weight chromosomal DNA under conditions that included a field angle of 120°.
and a voltage gradient of 6 V/cm. The enzymatic DNA of *Salmonella enterica*
Braenderup H9812 (ATCC BAA-664 provided by Centers for Disease Control and
Prevention, USA) was used as a molecular size marker. The gels were stained with
ethidium bromide and digitally photographed using the Gel Doc 1000 gel documentation
system (Bio-Rad) or scanned with Gel Compar Ver. 4.1 image analysis software (Applied
Maths, Kortrijk, Belgium). Finally, a total of 6 distinct SpeI-restriction PFGE patterns
(type I-VI) and 9 reproducible RAPD-types (A-I, band sizes ranging from 250 to 2500
base pairs) were detected among these isolates.

Melioidosis is a notifiable disease in Taiwan. All culture-confirmed cases of
melioidosis should be reported to the Taiwan CDC. According to Taiwan CDC data, a
total of 140 melioidosis cases were officially documented from 2000 to 2006 (Fig. 1).
Our environmental survey for the distribution of *B. pseudomallei* in soil revealed
that viable *B. pseudomallei* were only found in central (3.8%, 14/366, 95% CI =
0.025-0.059) and southern (12.6%, 48/381, 95% CI = 0.101-0.157) Taiwan. Highest
positive rate of *B. pseudomallei* was found in southern Taiwan. In addition, *B.
pseudomallei* genes were also detected by PCR across Taiwan. The prevalence was 1.3%
(4/306, 95% CI = 0.007-0.030), 10.1% (37/366, 95% CI = 0.079-0.131) and 19.4%
(74/381, 95% CI = 0.164-0.231) in northern, central and southern Taiwan, respectively.

same pattern as culture results which southern Taiwan had the highest positive rate (Fig.
1. 46.1% (53/115) of PCR positive soil samples in this study were not isolated to *B. pseudomallei*. By combining both culture and PCR detection results, *B. pseudomallei* soil prevalence was highest in southern Taiwan. The cases of melioidosis (0.03/100,000 in northern Taiwan, 0.29/100,000 in central Taiwan, and 1.98/100,000 in southern Taiwan) have significantly correlates with *B. pseudomallei* soil prevalence using logistic regression (culture method: $r^2=0.97$; PCR method: $r^2=0.86$). To determine the genetic relationship of environmental and clinical isolates, 47 environmental isolates and 6 clinical isolates were typed using PFGE and RAPD analysis (Table 1). Two clinical isolates (VGH11 and VGH14) had genetic patterns identical to some environmental isolates.

In this study, we systematically surveyed the geographical distribution of *B. pseudomallei* in Taiwan. Our results support the existence of autochthonous melioidosis in Taiwan. The soil prevalence in Taiwan as demonstrated in this study is comparable to melioidosis endemic regions elsewhere.

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Reference


Fig 1. The distribution of the case numbers, disease incidence rate, environmental isolation rates and PCR positive rates for melioidosis. Case numbers were obtained from official CDC Taiwan documents, Taiwan. Disease incidence rate was determined with occurrence of disease from 2000 to 2006. PCR positivity was defined as presence of both amplicons of 16S RNA and flagella gene in soil specimens. Soil samples were collected evenly every 5-10 Km along both sides of provincial roads (black line) throughout Taiwan. (Courtesy of Ming-chang Lin, reproduced with permission.)
Table 1. Molecular typing and their relatedness among soil and clinical isolates

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Isolated from</th>
<th>PFGE</th>
<th>RAPD</th>
<th>Molecular Typing related to Origins</th>
<th>Symptom of Patients</th>
</tr>
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<tbody>
<tr>
<td>KN34</td>
<td>C</td>
<td>V</td>
<td>H</td>
<td>NF†</td>
<td>NF†</td>
</tr>
<tr>
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<td>C</td>
<td>VI</td>
<td>I</td>
<td>NF</td>
<td>NF</td>
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<tr>
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<td>I</td>
<td>A</td>
<td>VGH14 blood</td>
<td>multiple organ abscesses</td>
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<tr>
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<td>S</td>
<td>II</td>
<td>C</td>
<td>NF</td>
<td>NF</td>
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<tr>
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<td>S</td>
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<td>D</td>
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<tr>
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<td>D</td>
<td>VGH11 blood</td>
<td>pneumonia, peritonitis</td>
</tr>
</tbody>
</table>

*: The bacteria were isolated from central (C) and southern (S) Taiwan.
†: The clinical strains were previously isolated from a melioidosis patient in KVGH (Kaoshiung Veterans General Hospital) in southern Taiwan (reference 3).
‡: NF, not found.