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Distribution of Melioidosis Cases and Viable *Burkholderia pseudomallei* in Soil: Evidence for Emerging Melioidosis in Taiwan▽

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A survey for the prevalence if *Burkholderia pseudomallei* in soil in Taiwan found that its incidence is comparable to that in other regions of the world where melioidosis is endemic. The presence of identical genetic patterns among the clinical and environmental isolates evaluated suggested a link between the pathogens present in contaminated soil and the emergence of indigenous melioidosis.

Melioidosis is a serious infection caused by *Burkholderia pseudomallei*, a soil-dwelling saprophyte mainly distributed between latitudes 20°N and 20°S (3). Human *B. pseudomallei* infections usually occur by inhalation or subcutaneous inoculation with contaminated materials (4). In Thailand, most patients with melioidosis are farmers who contract the bacteria as a result of high levels of exposure to *B. pseudomallei* during their agricultural work 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 in a traveler who had returned from the Philippines (6). From 1984 to 2000, only 20 cases of melioidosis were reported in Taiwan (5). These cases were categorized as being acquired during prior travels to areas of endemicity overseas rather than by indigenous acquisition, because the confirmed cases were rare and the pathogens were never isolated from the environment. However, the number of melioidosis cases suddenly increased in the Er-Ren River Basin in southern Taiwan after a typhoon followed by a flood in 2005 (8, 9). *B. pseudomallei* was isolated from agricultural crop soil, and the prevalence of *B. pseudomallei*-specific antibodies increased significantly among residents after the typhoon and flood incident (9). This raises the question of the extent to which *B. pseudomallei* is found in soil (natural environments) in Taiwan.

Thus, soil samples were collected from agricultural crop fields from October 2005 to March 2007. Each sampling site was 5 km or 10 km apart from the other sampling sites, and the sites were located throughout Taiwan. The agricultural crop fields were sampled by digging three separate holes. The digger was disinfected with 70% alcohol between soil collections. A total of 1,053 soil specimens were collected. Approximately 100 g of each sample was obtained at a depth of 30 to 60 cm, and 15 g was placed into 50 ml of Ashdown's broth in a 250-ml flask. The samples were processed, and the typical dry, wrinkled, violet-to-purple colonies of *B. pseudomallei* were enumerated (9). The clinical isolates ($n = 6$) obtained from patients with melioidosis who had never traveled overseas were also analyzed (1). Biochemical tests (API system; bioMérieux, Marcy l'Etoile, France) and molecular diagnostic tests (testing for the presence of the specific amplicons of the 16S RNA gene [243 and 405 bp] and flagellar gene [267 bp]) were used for confirmation of the presence of *B. pseudomallei* (9). The total DNA in the soil samples was isolated and purified with a soil genomic DNA extraction kit (GeneMark, Taiwan) and a purification kit (IsoQuick; ORCA Research Inc.), respectively. If the amplicons of both the 16S RNA and flagellar genes were amplified from total DNA from soil, it was concluded that *B. pseudomallei* was present in the soil sample.

The genetic relatedness among the clinical and environmental isolates was determined by randomly amplified polymorphic DNA (RAPD)-PCR and pulsed-field gel electrophoresis (PFGE) analyses. The RAPD-PCR primer used was GEN2-60-09 (5′-CCTCATGACC-3′), and a standardized protocol was followed (7). PFGE was performed in a CHEF-III DR system with 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* serovar Braenderup H9812 (ATCC BAA-664, provided by the Centers for Disease Control and Prevention, Atlanta, GA) was used as a molecular size marker. The gels were stained with ethidium bromide and digitally photographed with a Gel Doc 1000 gel documentation system (Bio-Rad) or were scanned with Gel Compar (version 4.1) image analysis software (Applied Maths, Kortrijk, Belgium). Finally, a total of six distinct SpeI restriction PFGE patterns (types I to VI) and nine reproducible RAPD types

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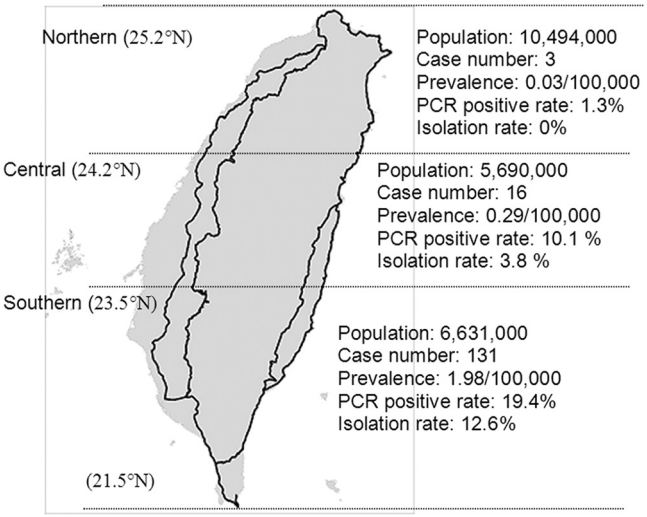

FIG. 1. Distributions of numbers of cases, disease incidence rate, environmental isolation rates, and rates of PCR positivity for melioidosis. Case numbers were obtained from official Taiwan CDC documents. The disease incidence rate was determined from the rates of occurrence of the disease from 2000 to 2006. PCR positivity was defined as the presence of both amplicons of the 16S RNA and flagellar genes in soil specimens. Soil samples were collected evenly every 5 to 10 km along both sides of provincial roads (black lines) throughout Taiwan. (Courtesy of Ming-Chang Lin, reproduced with permission.)

(types A to I; band size range, 250 to 2,500 bp) were detected among these isolates.

Melioidosis is a notifiable disease in Taiwan. All culture-confirmed cases of melioidosis should be reported to the Taiwan Centers for Disease Control (CDC). According to data from the Taiwan CDC, a total of 140 melioidosis cases were officially documented from 2000 to 2006 (Fig. 1).

TABLE 1. Molecular typing results and relatedness of soil and clinical isolates

Strain no.	Source location in Taiwan[a]	Molecular typing result		Relation to clinical strains[b]	Origin	Patient symptom(s)
		PFGE type	RAPD type			
KN34	C	V	H	NF[c]		
KN35	C	VI	I	NF		
KN37	C	I	A	VGH14	Blood	Multiple organ abscesses
KN03	S	II	C	NF		
KN04	S	IV	E	NF		
KN05	S	IV	F	NF		
KN06	S	IV	G	NF		
KN13	S	VI	I	NF		
KN17	S	V	H	NF		
KN18	S	I	A	VGH14	Blood	Multiple organ abscesses
KN23	S	II	B	NF		
KN28	S	III	D	VGH11	Blood	Pneumonia, peritonitis
KN58	S	I	A	VGH14	Blood	Multiple organ abscesses
KN59	S	III	D	VGH11	Blood	Pneumonia, peritonitis

[a] The bacteria were isolated from central (C) and southern (S) Taiwan.
[b] The clinical strains were previously isolated from a patient with melioidosis in the Kaoshiung Veterans General Hospital in southern Taiwan (1).
[c] NF, not found.

Our environmental survey for the distribution of *B. pseudomallei* in soil revealed that viable *B. pseudomallei* isolates were found only in central Taiwan (3.8%, 14/366 soil samples; 95% confidence interval [CI] = 0.025 to 0.059) and southern Taiwan (12.6%, 48/381 soil samples; 95% CI = 0.101 to 0.157) Taiwan. The highest rate of positivity for *B. pseudomallei* was found in southern Taiwan. In addition, *B. pseudomallei* genes were also detected by PCR of soil samples collected across Taiwan. The prevalence rates were 1.3% (4/306; 95% CI = 0.007 to 0.030), 10.1% (37/366; 95% CI = 0.079 to 0.131), and 19.4% (74/381; 95% CI = 0.164 to 0.231) in northern, central and southern Taiwan, respectively, which is the same pattern of results obtained by culture, by which southern Taiwan had the highest rate of positivity (Fig. 1). *B. pseudomallei* was not detected by culture in 46.1% (53/115) of the PCR-positive soil samples. By combining the results of both culture and PCR, the prevalence of *B. pseudomallei* in soil was the 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) had significant correlates with the prevalence of *B. pseudomallei* in soil, as determined by logistic regression (for culture method, $r^2 = 0.97$; for PCR method, $r^2 = 0.86$). To determine the genetic relationship of the environmental and clinical isolates, 47 environmental isolates and 6 clinical isolates were typed by PFGE and RAPD analyses (Table 1). Two clinical isolates (isolates VGH11 and VGH14) had genetic patterns identical to those of some of the environmental isolates.

In this study, we performed a systematic survey of the geographical distribution of *B. pseudomallei* in Taiwan. Our results support the existence of autochthonous melioidosis in Taiwan. The prevalence of *B. pseudomallei* in soil in Taiwan demonstrated in this study is comparable to that demonstrated in other regions where melioidosis is endemic.

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