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Melioidosis in Southern Vietnam: Clinical Surveillance and Environmental Sampling

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From 1992–1998, *Burkholderia pseudomallei* was isolated from only 9 (0.25%) of 3653 cultures of blood from febrile patients admitted to the Centre for Tropical Diseases in Ho Chi Minh City, an infectious disease referral center for southern Vietnam. Soil was sampled from 407 sites in 147 rice fields along the 5 major roads radiating from Ho Chi Minh City. *B. pseudomallei* was isolated from 73 sites (18%) in 39 rice fields (27%), but only 15 (21%) of the 71 isolates from 9 (6%) of 147 fields were the virulent L-arabinose (ara)–negative biotype. All except 1 of the fields with the ara-negative biotype were close to the homes of the patients with melioidosis. The low incidence of melioidosis in the provinces around Ho Chi Minh City may be explained by the restricted distribution of ara-negative *B. pseudomallei* in the soil in this area.

Melioidosis has been recognized as a clinical problem in Vietnam for >70 years [1]. It is a common cause of septicemia in Ubon Ratchathani, a city in northeast Thailand that is 500 km northwest of Ho Chi Minh City, Vietnam [2]; however, the infection has been diagnosed rarely in Vietnam in recent years [3, 4]. Melioidosis is caused by infection with the gram-negative bacterium *Burkholderia* (formerly *Pseudomonas*) *pseudomallei*, an environmental organism found in the soil of rice fields in Southeast Asia and northern Australia [5, 6]. There are 2 biotypes of *B. pseudomallei* that are defined by the ability to assimilate L-arabinose (ara) [7]. The ara-negative biotype is virulent and can be isolated from both clinical specimens and the environment, whereas ara-positive isolates are nonvirulent and are found only in the environment [7, 8]. Humans are usually infected by traumatic inoculation of soil into small wounds on the lower limbs while working. We report the incidence of melioidosis in an infectious disease hospital in Ho Chi Minh City and present the results of an environmental survey of rice fields in the surrounding region.

Methods

The Centre for Tropical Diseases in Ho Chi Minh City is a 500-bed infectious disease referral hospital for southern Vietnam. The

hospital admits 20,000–30,000 patients each year from Ho Chi Minh City and the surrounding provinces including the Mekong Delta. Since 1992, between 3500 and 5000 blood cultures have been performed annually. Blood specimens for culture (2–15 mL, depending on the age of the patient) were inoculated in 2- to 5-mL aliquots into 50 mL of brain-heart infusion broth (with added sodium polyanetholesulfonate). The broth was subcultured at 24 h, 48 h, and 7 days onto fresh sheep blood agar. From July 1997, the blood culture bottles were changed to BACTEC plus aerobic bottles and were incubated in a BACTEC 9050 automated culture system (Becton Dickinson, Sparks, MD). Bottles were subcultured when the machine gave a positive signal.

Cultures of other body fluids were performed by standard methods. Suspected isolates of *B. pseudomallei* that were oxidase-positive and gentamicin- and colistin-resistant were identified by the API 20NE System (bioMérieux, Basingstoke, U.K.).

A survey of rice fields covering a large area surrounding Ho Chi Minh City was undertaken. The area chosen covered >95% of the catchment area for referrals to the hospital. Soil samples were collected, as described elsewhere [5, 6] from rice fields along each major road radiating from Ho Chi Minh City at the beginning or end of the rainy season (June or November). Sites were chosen from fields ~5 km apart. At each site, soil samples were obtained from 2–4 places from rice fields on either side of the road; samples were taken at depths of 10–30 cm. Soil samples were processed the same day.

Approximately 100 g of soil from each site was mixed with 100 mL of sterile water in a plastic bag and left to sediment overnight. The upper layer of water (~20 mL) was transferred to a sterile container and shaken. One drop from a sterile pipette was subcultured directly onto modified selective Ashdown's agar containing gentamicin (8 mg/mL) and colistin (50 mg/L). A further 1 mL was diluted 1 : 10 in selective Galimand broth containing colistin (50 mg/L). The broth was subcultured after 48 h of incubation at 42°C onto modified Ashdown's agar.

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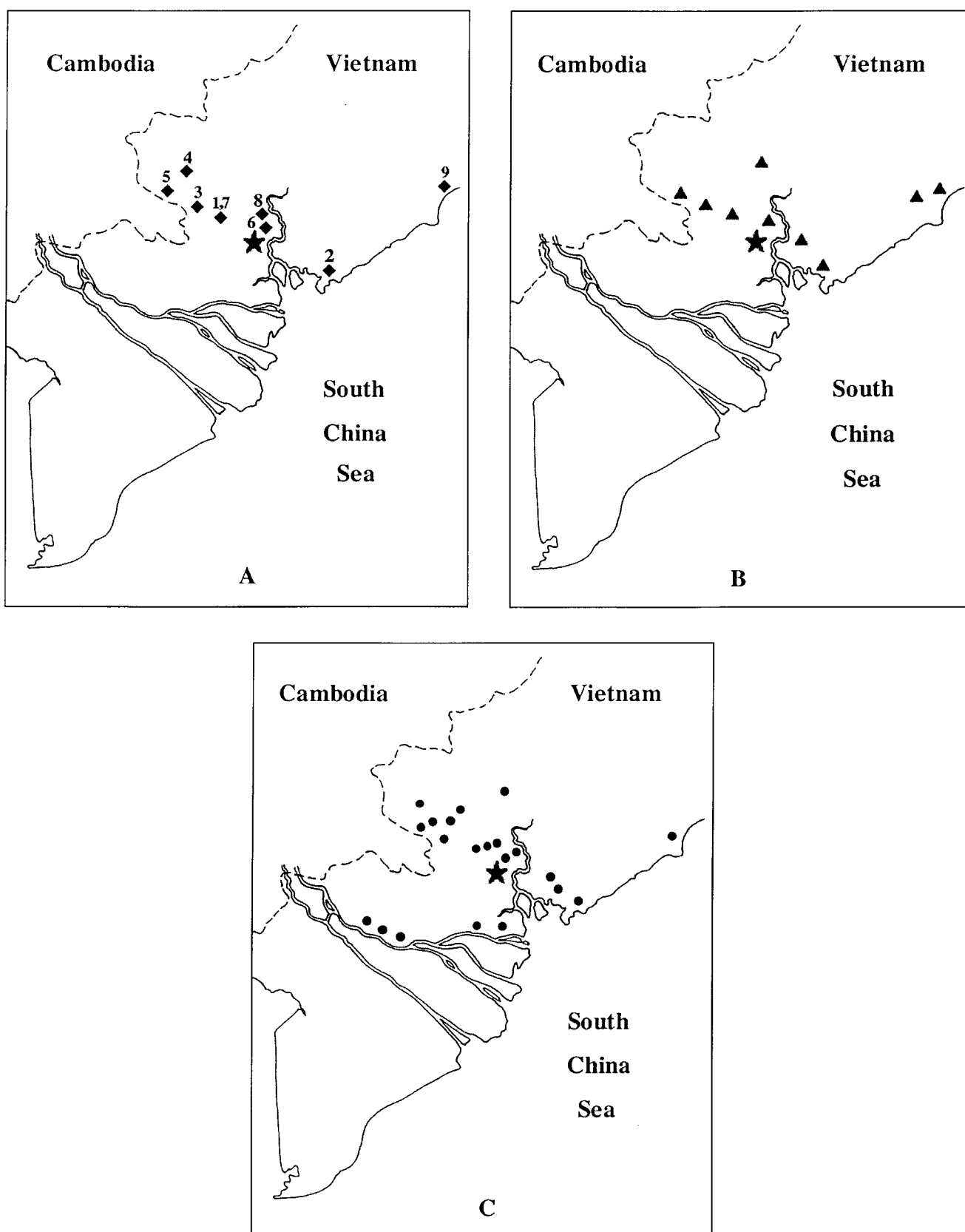


Figure 1. Map of southern Vietnam that shows catchment area for patient referrals to Centre for Tropical Diseases, Ho Chi Minh City (★, Ho Chi Minh City). *A*, Locations of homes of the 9 patients with melioidosis (◆). Numbers refer to order in which they presented to hospital. *B*, Locations of rice fields or groups of rice fields from which L-arabinose-negative *Burkholderia pseudomallei* was isolated from soil (▲). *C*, Locations of rice fields or groups of rice fields from which L-arabinose-positive *B. pseudomallei* was isolated from soil (●).

A small number of rice fields were selected randomly for more intensive study. Each of these rice fields was sampled at 7 sites. The soil samples were analyzed by quantitative culture; 100- μ L aliquots of the soil supernatant and 100 μ L of 2 serial 10-fold dilutions of the supernatant were inoculated onto plates with modified Ashdown's agar by means of a rotary spreader. Further aliquots of the soil supernatant (100 μ L and 1 mL) were added to 9 mL of modified Gallimand broth and subcultured onto modified Ashdown's media after 48 h.

All plates were incubated aerobically at 42°C for 4 days, and all morphologically distinct suspect colonies were investigated further. Isolates confirmed to be *B. pseudomallei* were tested for ara assimilation. On the quantitative culture plates, the number of colonies on each of the dilutions was used to calculate the number of bacteria per gram of the original soil sample. The counts of ara-negative *B. pseudomallei* were compared with the counts of ara-positive strains by the Mann-Whitney U test.

Results

From November 1992–November 1998, *B. pseudomallei* was isolated from 9 (0.25%) of 3653 patients admitted to our center with a positive blood culture. When *B. pseudomallei* was isolated from another sterile site, it was also always isolated from blood.

Soil samples were collected from 407 sites in 147 rice fields within a radius of \sim 100 km from Ho Chi Minh City. *B. pseudomallei* was isolated from 73 sites (18%) in 39 rice fields (27%). Fifteen (21%) of the 71 isolates from 9 (6%) of 147 fields were ara-negative. In each of 3 soil samples, there was a mixture of ara-positive and ara-negative isolates.

Quantitative cultures of samples from 7 sites in each of 18 rice fields were performed. Seven fields were negative. Between 1 and 5 sites were positive in the remaining fields. In 2 fields, only ara-negative strains were isolated, and in 4 fields, only ara-positive strains were isolated; however, in the remaining 5 fields, a mixture of ara-negative and ara-positive strains were isolated. The median number of *B. pseudomallei* was 90 cfu/g (interquartile range, 10–1000 cfu/g; range, 1–18,000 cfu/g). The median numbers of ara-negative and ara-positive strains were 70 cfu/g (interquartile range, 18–400 cfu/g; range, 10–2000 cfu/g) and 90 cfu/g (interquartile range, 3–1150 cfu/g; range, 1–18,000 cfu/g; $P > .5$).

Figure 1 shows the locations of the homes of the patients (figure 1A), the locations of the 9 fields from which ara-negative isolates were recovered (figure 1B), and the locations of the fields from which ara-positive isolates were recovered (figure 1C) are shown. Eight of the 9 fields with ara-negative isolates were close to the homes of the patients who had been admitted to the hospital with melioidosis.

Discussion

This study confirms that cases of melioidosis still occur in Vietnam. However, it does not appear to be a common cause

of sepsis in the provinces surrounding Ho Chi Minh City. It is much less common than in northeast Thailand, where *B. pseudomallei* is the cause of one-fifth of the cases of community-acquired septicemia [2]. Despite the fact that our center is a referral hospital, it is likely that the number of patients with melioidosis in this area of Vietnam was underestimated in this study. Patients with septicemic melioidosis may be admitted to the hospital and die rapidly if the correct diagnosis is not made. Culture facilities are unavailable in many of the district and provincial hospitals in the region. Furthermore, the amount of blood routinely obtained from each patient for culture at this hospital may sometimes be suboptimal.

B. pseudomallei was isolated from 27% of rice fields sampled within a radius of \sim 100 km from Ho Chi Minh City. This proportion is considerably less than in northeast Thailand around Ubon Ratchathani, where the organism may be isolated from 58% to 68% of rice fields [5, 6]. In a study of rice fields in northern Vietnam, *B. pseudomallei* was isolated from only 4 of 240 soil samples and 1 of 190 water samples, despite serological evidence of exposure to the bacterium in the local population in that area [4]. The median numbers of bacteria isolated from soil samples in northeast Thailand (where melioidosis is common) and central Thailand (where melioidosis is rare) were 230 and 10 cfu/mL, respectively [6]. Our results fall between these 2 values. Twenty-one percent of the soil isolates were ara-negative; this rate is less than in northeast Thailand, where 75% of soil isolates were ara-negative, but greater than in central Thailand, where all strains were ara-positive [8]. We isolated ara-negative and ara-positive strains from different sites in the same fields and, in 3 instances, from the same soil sample.

Most ara-negative isolates were found in fields close to where the patients lived. Although the low incidence of clinical melioidosis in southern Vietnam may be partly due to underdetection, it may also be due to restricted distribution of the virulent ara-negative strain of *B. pseudomallei* in the soil of rice fields in this area.

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References

1. Dance DAB. Melioidosis: the tip of the iceberg? Clin Microbiol Rev 1991;4: 52–60.
2. Chaowagul W, White NJ, Dance DAB, et al. Melioidosis: a major cause of community-acquired septicemia in northeastern Thailand. J Infect Dis 1989;159:890–9.

3. Hoa NTT, Diep TS, Wain J, et al. Community-acquired septicaemia in southern Viet Nam: the importance of multidrug-resistant *Salmonella typhi*. *Trans R Soc Trop Med Hyg* **1998**;92:503–8.
4. Phung LV, Quynh HT, Yabuuchi E, Dance DAB. Pilot study of exposure to *Pseudomonas pseudomallei* in northern Vietnam. *Trans R Soc Trop Med Hyg* **1993**;87:416.
5. Wuthiekanun V, Smith MD, Dance DAB, White NJ. Isolation of *Pseudomonas pseudomallei* from the soil in north-eastern Thailand. *Trans R Soc Trop Med Hyg* **1995**;89:41–3.
6. Smith MD, Wuthiekanun V, Walsh AL, White NJ. Quantitative recovery of *Burkholderia pseudomallei* from soil in Thailand. *Trans R Soc Trop Med Hyg* **1995**;89:488–90.
7. Smith MD, Angus BJ, Wuthiekanun V, White NJ. Arabinose assimilation defines a nonvirulent biotype of *Burkholderia pseudomallei*. *Infect Immun* **1997**;65:4319–21.
8. Wuthiekanun V, Smith MD, Dance DAB, Walsh AL, Pitt TL, White NJ. Biochemical characteristics of clinical and environmental isolates of *Burkholderia pseudomallei*. *J Med Microbiol* **1996**;45:408–12.