Melioidosis in Patients with COVID-19 Exposed to Contaminated Tap Water, Thailand, 2021

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In September 2021, a total of 25 patients diagnosed with COVID-19 developed acute melioidosis after (median 7 days) admission to a COVID-19 field hospital in Thailand. Eight nonpotable tap water samples and 6 soil samples were culture-positive for *Burkholderia pseudomallei*. Genomic analysis suggested contaminated tap water as the likely cause of illness.

Melioidosis is an infectious disease caused by the gram-negative bacillus *Burkholderia pseudomallei*, which is commonly present in soil and water in tropical countries (*1*,*2*). Naturally acquired infections result from skin inoculation, inhalation, or ingestion of *B. pseudomallei* (*1*). In 2020 and 2021, multiple COVID-19 field hospitals were set up in Thailand for cases of mild and moderate COVID-19 infection. We report 25 cases of acute melioidosis among patients diagnosed with COVID-19 who were being managed at a COVID-19 field hospital in Saraburi Province, central Thailand. Our study received ethical approval from the Committee of the Faculty of Tropical Medicine, Mahidol University (TMEC 24-006).

The Study

On September 8, 2021, the Department of Disease Control, Ministry of Public Health, Thailand, was alerted to a cluster of 20 patients with culture-confirmed melioidosis (case nos. 1–20; Table, https://wwwnc.cdc.gov/EID/article/30/4/23-1476-T1.htm). The 20 patients had been admitted to a single field hospital in Saraburi Province, which had been designated a treatment facility for COVID-19, and were transferred to Kaeng Khoi Hospital, Saraburi Province, because they developed fever or pneumonia. Previously, Saraburi Province had diagnosed ≈8–12 culture-confirmed melioidosis cases per year (*2*). The outbreak investigation team suspected that nonpotable tap water (NPTW) was the source of infection because there were no other apparent sources (Appendix, https://wwwnc.cdc.gov/eid/article/30/4/23-1476-App1.pdf). The initial response included the immediate transfer of patients with diabetes and those who had received steroid therapy for COVID-19 to other hospitals, followed by re-emphasizing to staff the recommended prevention strategies for melioidosis (*3*). The recommendations included avoiding direct exposure to soil and environmental water and drinking only boiled or bottled water.

Health officials immediately planned and conducted an environmental investigation. During September 10–16, 2021, the outbreak investigation team collected samples from the field hospital, including

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8 commercially bottled drinking water (CBDW) samples (500 mL–1 L), 37 NPTW samples from 10 locations (1-L samples; 3–4 samples per location at different time points), and 50 soil samples (100 g per sample) (Appendix). We isolated *B. pseudomallei* from environmental samples according to previously described methods (*4*,*5*). None of the CBDW samples, 6 (12%) of 50 soil samples, and 8 (22%) of 37 NPTW samples (from 4 locations) were culture-positive for *B. pseudomallei*. The median quantitative count of *B. pseudomallei* in NPTW was 24.5 CFU/L (range 4–58 CFU/L) and in soil was 82 CFU/g (range <1–119 CFU/g). The outbreak investigation team found that the chlorination system for NPTW was not well maintained. Patients reported that they drank only CBDW and never drank NPTW, which was used for other domestic purposes, such as brushing their teeth, rinsing their mouths, and showering. The chlorination system was successfully repaired, and chlorine levels were maintained >1 ppm beginning on September 10. A further 5 melioidosis cases were identified, all of whom had been admitted before September 10. No new melioidosis cases among those who had stayed at the field hospital were reported after September 16.

Of the 25 patients diagnosed with melioidosis (Table), 12 (48%) were female and 13 (51%) male; median age was 59 (interquartile range 56–62, range 34–73) years. All patients had received a diagnosis of COVID-19, confirmed by PCR during August 16–29, 2021, and had been admitted to the field hospital in August 22–September 2, 2021. A total of 15 (60%) patients had diabetes, and all 25 (100%) patients had received steroids as part of their COVID-19 treatment. The date range of onset of symptoms attributed to melioidosis was September 1–11. The median time from admission to the field hospital to the onset of the melioidosis symptoms was 7 (interquartile range 5–9, range 4–20) days.

The most common clinical manifestations of melioidosis among patients in this cluster were secondary bacterial pneumonia (n = 22 patients [88%]) and fever (n = 15 [60%]) (Table). Clinical specimens that were culture-positive for *B. pseudomallei* were blood (n = 24 [96%]) and sputum (n = 1 [4%]). In-hospital mortality for patients we studied was 32% (8/25). Of the fatal cases, 3 patients (case nos. 2, 4, and 8) died without receiving ceftazidime or meropenem, which are recommended parenteral antibiotics for treatment of melioidosis. A total of 17 (68%) cases completed ≥10 days of parenteral ceftazidime or meropenem and subsequently received a course of oral eradicative treatment.

We confirmed the first 20 clinical isolates and all environmental isolates as *B. pseudomallei* at the Mahidol-Oxford Tropical Medicine Research Unit laboratory, Bangkok, by using a combination of colony morphology on Ashdown agar, latex agglutination test, arabinose assimilation test, and antimicrobial susceptibility tests. Testing revealed that all clinical and environmental isolates were susceptible to ceftazidime, meropenem, and trimethoprim/sulfamethoxazole. We performed whole-genome sequencing on 19 clinical isolates, 8 NPTW isolates, and 6 soil isolates (1 colony per patient or sample). We excluded 1 clinical isolate from analysis because of low sequencing depth of 7.5×. We deposited sequences in the European Nucleotide Archive (https://www.ebi.ac.uk/ena) (accession numbers in Appendix Table). We mapped isolates to the K96243 reference genome and used variant calls to construct a phylogeny after masking recombinant fragments, repetitive regions, and known *B. pseudomallei* genomic islands (*6*). We used genome assemblies to call multilocus sequence types (STs).

We categorized the isolates by both phylogenetic and multilocus sequence typing, and they clustered consistently into 4 groups. The largest cluster was ST689 and included all 19 blood and sputum samples, as well as 6 of 8 NPTW samples (Figure). The remaining soil and NPTW isolates formed 3 separate clusters (ST107, ST303, and ST315). Within the ST689 cluster, the isolates were closely related but not identical (12–98 SNP differences between isolates). The NPTW isolates were interspersed with clinical isolates in this cluster, suggesting that contaminated NPTW was a possible source of infection for these patients. Dating analysis was not feasible because of the absence of clock signals in the phylogeny.

Conclusions

Our study highlights that patients with viral infections (e.g., COVID-19) may be at risk for infection and death caused by melioidosis if exposed to NPTW contaminated by *B. pseudomallei*. Diabetes mellitus and conditions that impair innate and adaptive immune responses, particularly steroid use, are important risk factors for melioidosis (*1*). Diabetes mellitus is also a risk factor for COVID-19, and steroid treatment is recommended for patients with COVID-19 pneumonia (*7*). Therefore, unsurprisingly, co-infections with COVID-19 and *B. pseudomallei* have been reported occasionally (*8*,*9*; D. Chit Yee et al., unpub. data, https://wellcomeopenresearch.org/articles/7-160), including 1 of the 4 patients detected during the multistate outbreak of melioidosis caused by an imported aromatherapy spray in the United States (*10*), and now

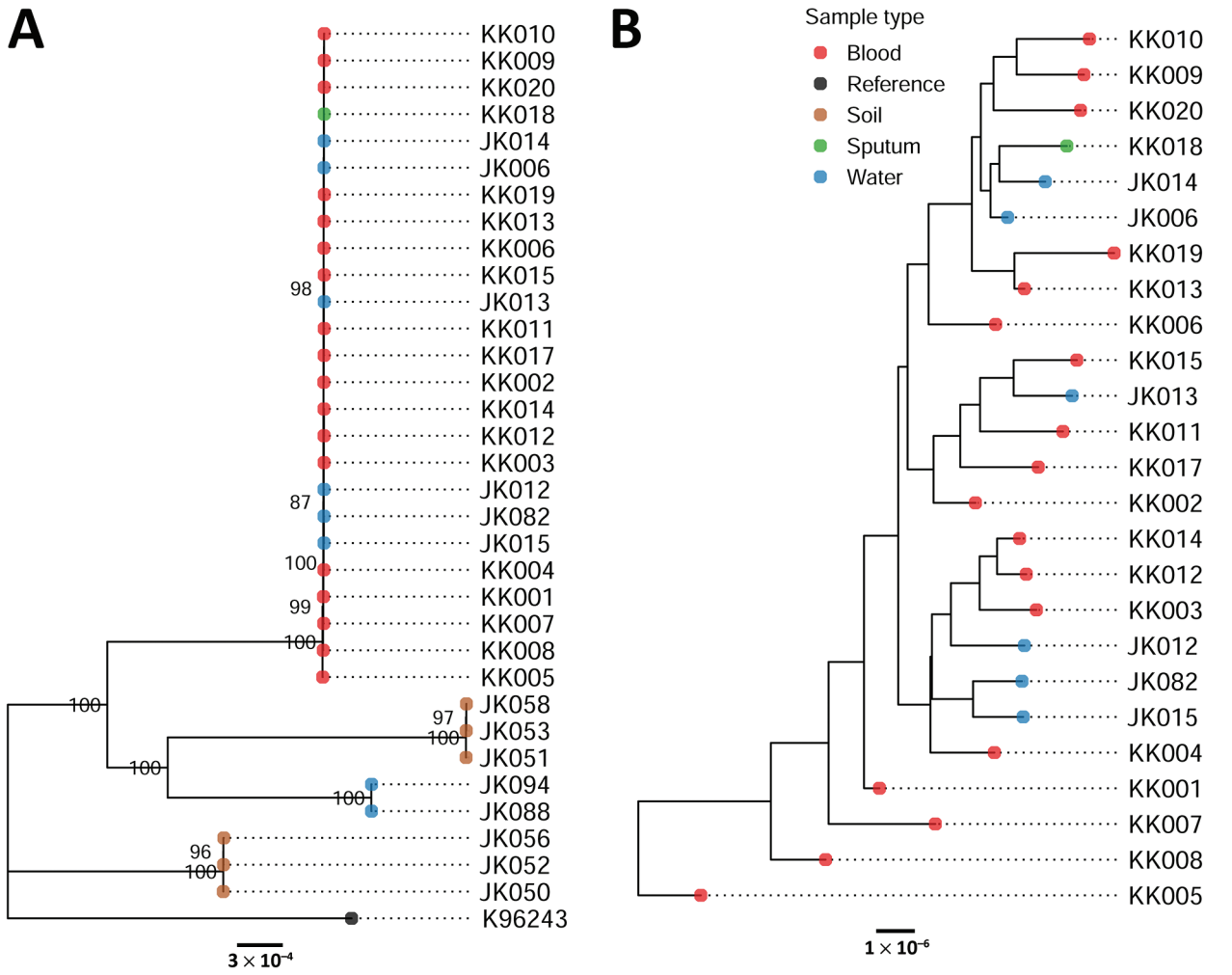

Figure. Information on 25 patients with COVID-19 who developed acute melioidosis in Saraburi Province, Thailand, 2021. A) Phylogenic tree of 19 clinical isolates and 14 environmental isolates. The K96243 reference strain is used to root the tree. B) Tree showing only the clinical and environmental isolates within the ST689 cluster. Scale bar shows number of nucleotide differences.

this cluster. Previous reports of co-infection with influenza A (*11*,*12*) or COVID-19 (*9*) and *B. pseudomallei* suggested that melioidosis could be reactivated from a latent focus following viral infection. However, the timeline of the cluster, the identified source, and genomic analysis suggest that the patients in this cluster represented recently acquired secondary infections after COVID-19. The route of infection in this cluster was probably skin exposure to contaminated NPTW at a high-infecting dose, although ingestion or inhalation are also possible.

An unknown proportion of melioidosis patients in melioidosis-endemic areas could be related to exposure to contaminated NPTW. More studies on the effects of *B. pseudomallei*-contaminated NPTW and its disinfection (*13*) in melioidosis endemic areas are required. Because general recommendations for melioidosis prevention (*3*) do not emphasize the disinfection of NPTW, those recommendations may be inadequate and should be revisited.

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Appendix

Our study received ethical approval from the Committee of the Faculty of Tropical Medicine, Mahidol University (TMEC 24–006). Individual consent was not sought from the patients as this was a retrospective study, and the Ethical and Scientific Review Committees approved the process.

On 8th September 2021, the Department of Disease Control, Ministry of Public Health, Thailand was alerted to a cluster of 20 patients with culture-confirmed melioidosis. Using initial information obtained from the field hospital, Kaeng Khoi Hospital and Saraburi Hospital, the outbreak investigation team suspected that nonpotable tap water (NPTW) was the most likely source of infection. This was because (a) all COVID-19 patients in the cluster had been quarantined in five buildings in the field hospital, all buildings had concrete floors, they had not been exposed to soil, rice fields or surface water before the onset of symptoms, and, therefore, skin exposure to environmental soil or water was not primarily suspected as the route of infection; (b) there had been no storms or heavy rainfall before the onset of symptoms, and, therefore, inhalation of environmental aerosols was not primarily suspected as the route of infection; (c) all COVID-19 patients in the field hospital drank commercial bottled drinking water (CBDW) which was donated to the field hospital, CBDW was also donated to other field hospitals but there were no melioidosis cases from those field hospitals, and, therefore, CBDW was not primarily suspected as the source of infection; (d) food was cooked and prepared for the field hospital according to hospital standards; therefore, food was not primarily suspected as the source of infection; and (e) all patients exposed to NPTW for their daily activities such as brushing their teeth, rinsing their mouths and showering, there were no records of quality testing of NPTW at the field hospital, and, consequently, NPTW was suspected as the most likely source

of infection. Patients reported that they drank only CBDW and never drank NPTW. There were shower facilities in the field hospital that used NPTW, but there were no bathtubs.

The initial response included transferring diabetic patients and those who had received steroid therapy for COVID-19 to other hospitals immediately. This was because diabetes and steroid use are known as important risk factors for melioidosis in Thailand. A case-control study in 1999 in Thailand found that the use of herbal medicine, which might contain steroids, was more common in melioidosis patients that that in patients with non-infectious diseases (10% versus 5%) (*1*). Steroid use was also observed as a risk factor in several melioidosis case reports (*2*). A case-control study in 2013 in Thailand found that steroid intake was independently associated with melioidosis (adjusted conditional odds ratio 3.1; 95% confidence interval 1.4–6.9, p = 0.006) (*3*).

An environmental investigation was immediately planned and conducted. On 10th September 2021, 50 soil samples (1 sample/sampling point; 100 g/sample) were collected. Six sampling points were around building A, six sampling points around building B, six sampling points around building C, six sampling points around building D, 10 sampling points around building E, four sampling points around building F, seven sampling points around building I, two sampling points around a building that was set up for chest radiography, two sampling points around the parking area, and one sampling point in front of building Z. We anonymized the names of the buildings. All COVID-19 patients stayed in buildings A to E. On 10th September, eight CBDW and 17 NPTW samples (one liter/sample) were also collected. The 17 NPTW samples were collected from taps in buildings A, B, C, D, E, F, I and another three tap locations within the field hospital. NPTW samples were collected at noon and in the evening from each tap. The evening samples were not obtained from the taps in buildings B, C and D. On 13th September, another 10 NPTW samples were collected from each tap. On 16th September, another 10 NPTW samples were collected from each tap.

None of the CBDW samples, six of 50 soil samples (12%) and eight of 37 NPTW samples (22%) (from four locations) were culture positive for *B. pseudomallei*. The six soil samples culture positive for *B. pseudomallei* were collected from six sampling points around building E. The eight NPTW samples culture positive for *B. pseudomallei* were collected from

the tap in building E (four samples), the tap in building F (three samples) and the tap in building I (one sample).

Of the eight fatal cases, three died without receiving ceftazidime or meropenem, which are recommended parenteral antibiotics for treatment of melioidosis (Case Nos. 2, 4 and 8). Those three cases died before the results of blood cultures were available. For example, Case No. Two had blood specimens collected for bacterial culture on 1st September and died on the same day. Case No. Eight had blood specimens collected for culture on 2nd September and died on the same day. In Thailand, it is not uncommon that melioidosis patients die before the results of bacterial culture are available (*4*). The median time between taking the blood culture and the sample flagging as positive was 1 day (interquartile range 1–2 days) (*5*,*6*), and it would take another 1 or 2 calendar days for microbiology laboratories to subculture and confirm that the isolate was *B. pseudomallei*. Therefore, ceftazidime or meropenem are recommended for empirical treatment for community-acquired sepsis patients in melioidosis-endemic areas (*7*). Nonetheless, Saraburi province is in central Thailand and the incidence of melioidosis is relatively low compared to that in northeast Thailand (*8*). Therefore, clinicians did not suspect melioidosis during the very early phase of the cluster (i.e., 1st-3rd September). After the confirmation of the bacterial culture results of the first few cases, ceftazidime or meropenem were then used for empirical treatment among COVID-19 patients who were transferred from the field hospital with acute sepsis or pneumonia.

The route of infection in this cluster was probably skin exposure to contaminated NPTW at a high infecting dose, although ingestion or inhalation are also possible. In this cluster, patients had a high proportion of bacteraemia, pneumonia, and mortality. This could be associated with underlying COVID-19 and diabetes, immunosuppression caused by the steroid therapy, and other confounding factors. Diarrhea was noted as one of the clinical presentations in Cases 4, 8 and 18. This could be because (a) contaminated NPTW was ingested while rinsing mouths or showering and gastrointestinal tract infection was primarily involved, or (b) *B. pseudomallei* was disseminated to the gastrointestinal tract after skin exposure to contaminated NPTW.

To our knowledge, this is the largest cluster of *B. pseudomallei* in humans acquired from contaminated NPTW. Acquisition of *B. pseudomallei* infection in humans from contaminated NPTW has previously been reported from Australia (*9–11*) and Thailand (*12*,*13*). A study

previously reported 159 infected swine from 1981 to 1983 in southern Queensland (*14*), where the water supply was suspected as the source of infection although this could not be confirmed.

The study has some limitations. First, the study does not have additional detailed information, such as individual levels of exposure to NPTW, COVID-19 presentations and symptoms and dosages of steroid use. According to the COVID-19 guidelines in Thailand, none of the COVID-19 patients admitted or transferred to the field hospital had symptoms of severe COVID-19, such as high fever (≥39°C), requirement for oxygen supplementation, severe co-morbidities, or other conditions that attending physicians considered to necessitate hospital admission. Second, the study team does not have information on other COVID-19 patients who were admitted to the field hospital and did not develop acute melioidosis. Therefore, we could not evaluate odds ratios or hazard ratios for the risk of acquiring melioidosis.

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Appendix Table. Accession numbers of 18 blood isolates, 1 sputum isolate, 8 water isolates, and 6 soil isolates of *Burkholderia pseudomallei* sequenced in this study

Sample ID	SRA accession
JK052	ERS14632051
JK053	ERS14632052
JK058	ERS14632053
JK094	ERS14632057
JK006	ERS14632043
JK012	ERS14632045
JK013	ERS14632046
JK014	ERS14632049
JK015	ERS14632047
JK050	ERS14632050
JK051	ERS14632048
JK056	ERS14632054
JK082	ERS14632055
JK088	ERS14632056
KK001	ERS14632058
KK002	ERS14632059
KK003	ERS14632060
KK004	ERS14632061
KK005	ERS14632062
KK006	ERS14632063
KK007	ERS14632064
KK008	ERS14632066
KK009	ERS14632065
KK010	ERS14632067
KK011	ERS14632068
KK012	ERS14632070
KK013	ERS14632069
KK014	ERS14632072
KK015	ERS14632071
KK016	ERS14632073
KK017	ERS14632077
KK018	ERS14632076
KK019	ERS14632075
KK020	ERS14632074