

Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

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3 NEJM Supplemental

4 Imported Aromatherapy Spray Associated Multi-State Outbreak of Melioidosis 2021 – United States

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45 2. Methods

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56 Isolates of *B. pseudomallei* from patients (i.e. KS2021a, TX2021a, MN2021a, and GA2021a) were submitted
57 by the state health departments to the CDC for analysis. In collaboration with CDC, Health Departments in
58 Kansas, Texas, Minnesota, and Georgia interviewed patients, if possible, and family members to determine
59 potential exposures to *B. pseudomallei*. Environmental and/or household products were tested from each of
60 the 4 patients' homes. Items selected were diverse based on possible topical, ingestion, or inhaled exposure.
61 Over 200 samples were tested and included swabs from and samples of aerosol spray products, aquariums,
62 food, soaps, hand sanitizer, household cleaning products, insecticides, personal care products, medications,
63 pet supplies, soil, vitamins, and water. Samples were tested for the presence of *B. pseudomallei* using
64 consensus guidelines for environmental sampling with some modifications including the use of the Qiagen
65 EZ1 advanced XL instrument with the EZ1 advanced XL tissue card and EZ1 DNA tissue kit¹⁻³.

66 During the course of the investigation, a bottle of Better Homes and Gardens-branded Essential Oil and
67 Semi-precious Stone Infused Aromatherapy Room Spray "Lavender & Chamomile" found in the home of
68 patient 4 was found to be positive for *B. pseudomallei* and an isolate (GA2021_Spray_1A) from it was used
69 for further analysis. Additional testing of other bottles from the same product line is ongoing. The isolates
70 underwent whole genome sequencing (WGS). Sequences were deposited in NCBI under PRJNA763213.
71 Isolates were analyzed as previously described for multilocus sequence typing (MLST) and phylogeography⁴⁻
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74 3. Laboratory Analysis

75 Analysis of single nucleotide polymorphisms (SNPs) showed that all five isolates differentiated by < 10 SNPs,
76 indicating clonality and suggesting that all patients were exposed to the same aromatherapy product or
77 different products sharing the same contaminated component used for the spray bottle associated with
78 patient 4. We have designated the name “ATS2021” (i.e. Aromatherapy Spray 2021) for this strain. Strain
79 ATS2021 was not a close match to any of the 1,696 *B. pseudomallei* genomes publicly available in RefSeq,
80 although it clustered closest to examples from India and Sri Lanka (Figure 3 of main text). The contaminated
81 spray bottle found in the home of patient 4, Better Homes and Gardens-branded Essential Oil Infused
82 Aromatherapy Room Spray with Gemstones “Lavender & Chamomile”, was imported from India. The strain
83 yielded a novel sequence type, ST-1941, by MLST⁷.

84 4. Treatment Recommendations

85 Treatment for melioidosis with intravenous antibiotics (e.g., ceftazidime or meropenem) for at least two
86 weeks is recommended. Intravenous treatment may be extended for up to eight weeks depending on
87 response. To prevent relapse intravenous treatment is followed by oral trimethoprim-sulfamethoxazole
88 (TMP/SMX) for three to six months. Amoxicillin/clavulanic acid or doxycycline may be used in patients with a
89 contraindication to, or who cannot tolerate, TMP/SMX⁸.

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