Genomic Diversity of *Burkholderia pseudomallei* Isolates, Colombia

Appendix

Materials and Methods

Through laboratory-based surveillance activities, 11 *Burkholderia pseudomallei* isolates were received by the microbiology group of the Instituto Nacional de Salud in Colombia during 2016–2017. Cultures from blood, sputum, urine, abscesses, and throat swabs generated as part of routine diagnostic procedures were processed according to the protocols of the clinical laboratory of each hospital. We performed preliminary identification of isolates and susceptibility tests using a VITEK 2 (Biomerieux, https://www.biomerieux-usa.com). Isolates that we identified as *Burkholderia* spp., oxidase positive, gram-negative, and non-*Pseudomonas aeruginosa* bacteria, were further tested by MALDI-TOF MS (Bruker, https://www.bruker.com) (1).

Six isolates presumptively identified as *B. pseudomallei* or *Burkholderia* spp. were sent to the U.S. Centers for Disease Control and Prevention (CDC) for confirmatory testing, whole genome sequencing, and genetic analysis. DNA from an additional 5 *B. pseudomallei* isolates were also sent to CDC for sequencing and genetic analysis. Colombia has previously reported 20 cases as sporadic, isolated events in a few geographic areas. The departments with melioidosis cases from this study are noted on the map in the Appendix Figure. Accounts of previous cases of melioidosis in Colombia, including maps, have been published elsewhere (2–10).

We extracted DNA using the Maxwell RSC Cultured Cells DNA kit on the Promega Maxwell RSC Instrument per the manufacturer’s instructions (https://www.promega.com) or extracted it using a QIAGEN DNeasy Blood & tissue kit (https://www.qiagen.com) from pure overnight culture, according to the manufacturer’s instructions. We quantified DNA concentration and spectrum ratios using a ThermoFisher
Qubit v4.0 fluorometer (https://www.thermofisher.com). We eluted samples in PCR-grade water and RNase A, filtered through a 0.1 µm filter, and checked for sterility before whole genome sequencing (11).

We determined isolate sequences from paired-end Illumina reads which were generated on an Illumina MiSeq or iSeq 100 (https://www.illumina.com). We sheared genomic DNA to a mean size of 600 bp using a Covaris LE220 focused ultrasonicator (https://www.covaris.com). We cleaned DNA fragments with a Beckman Coulter Ampure system (https://www.beckmancoulter.com) and used them to prepare dual-indexed sequencing libraries using NEBNext Ultra library prep reagents (New England Biolabs, https://www.neb.com) and barcoding indices synthesized in the CDC Biotechnology Core Facility for the genomes run on the MiSeq. Libraries were analyzed for size and concentration, pooled, and denatured for loading onto the flow cell for cluster generation. We used 2 × 250 bp cycle paired-end sequencing kits to perform sequencing for the Illumina MiSeq. We used a Nextera Flex kit (Illumina) to produce libraries for the iSeq 100 runs, which we performed using 2 × 150 bp cycle paired-end sequencing kits. On completion, sequence reads were filtered for read quality, base called, and demultiplexed using bcl2fastq, version 2.19 (Illumina). We generated assemblies as previously described and assessed them with QUAST v5.0 (https://github.com; 12,13). Features of the genome assemblies are noted in Appendix Table 1.

We submitted genomes to the B. pseudomallei MLST website (http://pubmlst.org/bpseudomallei) to identify the sequence types or assign new sequence type identifiers, as needed (14,15). We analyzed core SNPs for the genomes from Colombia using Parsnp in the Harvest 1.3 suite (https://github.com) along with a reference panel previously described, plus genomes associated with the Western Hemisphere that have recently become available (11,16–19). The Colombian genomes had an average of 3,822 SNPs in nonprotein-encoding (intergenic) positions compared with K96243; 2.1 × more SNPs were observed in genes that had no predicted amino acid changes (Appendix Table 2). The dendrogram was generated in MEGA 7 (https://www.megasoftware.net) (20). SNP effects of the Colombian isolates compared with the K96243 reference strain were predicted with SnpEff v4.3t (https://github.com; 21).
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Appendix Table 1. General features of Colombian genome assemblies.

| Sample | Contigs* | Total length (bp) | Largest contig, bp | GC, %† | N50‡ | L50§ |
|--------|----------|-------------------|-------------------|--------|-------|------|
| B107   | 289      | 7,125,249         | 137,454           | 68.08  | 43,818 | 53   |
| B108   | 463      | 7,106,012         | 99,738            | 68.04  | 28,313 | 76   |
| B109   | 444      | 7,134,078         | 134,685           | 68.06  | 31,337 | 68   |
| B196   | 585      | 7,226,750         | 108,429           | 67.76  | 24,102 | 87   |
| B197   | 466      | 7,204,391         | 117,090           | 68.01  | 28,036 | 78   |
| B198   | 394      | 7,008,319         | 120,168           | 68.22  | 34,483 | 62   |
| B199   | 259      | 7,040,139         | 292,250           | 68.25  | 51,517 | 39   |
| B255   | 536      | 7,204,800         | 98,505            | 67.97  | 27,345 | 81   |
| B308   | 311      | 7,016,254         | 170,164           | 68.24  | 44,603 | 49   |
| B309   | 296      | 7,018,475         | 195,367           | 68.25  | 48,879 | 44   |
| B310   | 321      | 7,086,990         | 152,984           | 68.14  | 40,384 | 52   |
| B411   | 357      | 7,026,297         | 126,891           | 68.19  | 40,276 | 55   |

*No. of contiguous sequences assembled from short raw Illumina sequences
†Percentage of a genome assembly containing Guanine and Cytosine nucleotides
‡Length of the smallest contig, which together with larger contigs comprise half of the total assembly size
§Smallest contig quantity to make up 50% of the total assembly size

Appendix Table 2. Predicted mutation consequences of SNPs observed in the Colombian isolates compared with the reference strain K96243 (GCA 000959285.1).

| Sample | Synonymous | Missense | Intergenic | Noncanonical start codon | Start codon lost | Stop codon gained | Stop codon lost |
|--------|------------|----------|------------|--------------------------|-----------------|------------------|----------------|
| B107   | 7920       | 9400     | 3799       | 15                       | 33              | 307              | 0              |
| B108   | 7957       | 9386     | 3872       | 15                       | 29              | 302              | 0              |
| B109   | 7935       | 9307     | 3807       | 12                       | 31              | 307              | 0              |
| B196   | 7982       | 9445     | 3898       | 14                       | 35              | 319              | 0              |
| B197   | 7929       | 9415     | 3759       | 15                       | 32              | 289              | 0              |
| B198   | 7993       | 9376     | 3860       | 16                       | 29              | 312              | 0              |
| B199   | 7872       | 9271     | 3687       | 12                       | 33              | 314              | 0              |
| B255   | 7924       | 9387     | 3796       | 15                       | 33              | 307              | 0              |
| B308   | 7908       | 9373     | 3833       | 15                       | 33              | 306              | 0              |
| B309   | 7990       | 9386     | 3873       | 13                       | 30              | 310              | 0              |
| B310   | 7920       | 9400     | 3801       | 15                       | 33              | 307              | 0              |
| B411   | 7984       | 9518     | 3873       | 13                       | 30              | 310              | 0              |
Appendix Figure. Map of Colombia showing number of melioidosis cases by department.