Fatal Case of Diphtheria and Risk for Reemergence, Singapore

Technical Appendix

Whole-Genome Sequencing Analysis

For preparation of whole-genome sequence libraries, purified DNA was quantified and processed by using the Nextera XT Library Preparation Kit (Illumina, Inc., San Diego, CA, USA) for generation of sequencing libraries as per the manufacturer's instructions. Multiplex paired-end libraries (2×250–300 bp) were sequenced on an Illumina Miseq instrument and the Miseq v3 protocol. In silico multilocus sequence typing (MLST) based on the *Corynebacterium diphtheriae* MLST scheme (https://pubmlst.org/cdiphtheriae/info/protocol.shtml) was inferred from the whole-genome sequence by using SRST2 (*1*). MLST sequences were obtained by read mapping genome data to a database of reference allele sequences (downloaded from PubMLST) to extract a consensus sequence for each allele for each isolate. Multiple alignment and phylogenetic analysis was performed by using MEGA7 (*2*). The mlst tool (https://github.com/tseemann/mlst) was used to deduce the sequence type (ST) for 199 publicly available *C. diphtheriae* genomes downloaded from Genbank, of which 4 were found to belong to ST50. Of the 4 ST50 publicly available genomes, 3 were submitted from India (GCA_001723455.1, GCA_ 001935895.1, and GCA_001981275.1) and 1 from Germany (GCA_000255215.1). The genome submitted from Germany was originally obtained from the Centers for Disease Control and Prevention (*3*). Rapid core genome single-nucleotide polymorphism analyses were performed by using parsnp (*4*).

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