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ESBL-Producing and Macrolide-Resistant *Shigella sonnei* Infections among Men Who Have Sex with Men, England, 2015

Technical Appendix

Detailed Methods

Public Health England conducted whole-genome sequencing of ≈70% of *S. sonnei* isolates voluntarily referred from hospital laboratories. In brief, DNA is extracted from cultures of *Shigella sonnei* isolates for sequencing on the HiSeq 2500 (Illumina, San Diego, USA). High-quality reads are mapped to the *S. sonnei* reference genome Ss046 (GenBank accession no. NC_007384.1) by using the Burrows-Wheeler Aligner's Smith-Waterman Alignment algorithm (*1*). Single nucleotide polymorphisms (SNPs) are identified by using the Genome Analysis Toolkit (*2*) in unified genotyper mode. Core genome positions with a high-quality SNP (>90% similarity, minimum depth [minimum number of reads needed to call a genotype] ×10, genotype quality >30) in at least 1 isolate are extracted, and maximum likelihood phylogeny is derived by using RaxML (*3*).

When necessary to elucidate plasmid sequence, DNA is extracted using the molecular Invisorb Spin Mini Kit (Stratec Biomedical) and is diluted to a concentration of 1 μg of genomic DNA in 50 μL of water. A MinION library (Oxford Nanopore Technologies, Oxford, UK) is prepared by using the SQK-MAP006 genomic sequencing kit (Nanopore), according to the manufacturer's instructions, and sequencing is performed on an Mk1 MinION device (Nanopore) with an Mk1 flow cell. A FASTA file of the readings is extracted with poretools (*4*) and SPAdes version 3.6.2 (*5*), which is used to produce a hybrid.

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