Gonorrhoea treatment failure caused by a *Neisseria gonorrhoeae* strain with combined ceftriaxone and high-level azithromycin resistance, England, February 2018: Supplement

**Methods**

**Microbiology**

Testing for *N. gonorrhoeae* was undertaken by nucleic acid amplification test (NAAT) using the BD ProbeTec GC Qx amplified DNA assay and confirmed by BD Max GC real-time PCR assay (BD, Wokingham, United Kingdom[UK]) and culture on Lysed GC selective agar (Oxoid, Basingstoke, UK) at Oxford University Hospitals, UK. Antimicrobial susceptibility testing was undertaken by M.I.C.Evaluator Strips (Oxoid, Basingstoke, UK), according to the manufacturer’s instructions, with results confirmed using Etest (BioMérieux, Marcy l’Etoile, France) at Public Health England, Colindale, UK and the WHO Collaborating Centre for Gonorrhoea and other STIs, Sweden. European Committee on Antimicrobial Susceptibility Testing resistance breakpoints were used.
Sequencing

DNA was extracted from cultured isolates for long-read sequencing using Genomic Tip 100/G (Qiagen, Manchester, UK) and for short-read sequencing using QuickGene (AutoGen Inc., Holliston, MA). Isolates underwent short- and long-read sequencing using MiSeq (Illumina Inc., San Diego, CA) and Oxford Nanopore Technologies (ONT, Oxford, UK) MinION respectively. DNA libraries were prepared using Nextera and ONT library kits according to the manufacturer’s instructions. ONT base calling was performed using Guppy v.0.5.1.

Hybrid assembly of Illumina and filtered ONT reads, using Filtlong v.0.2.0 (with settings filtlong --min_length 1000 --keep_percent 90 --target_bases 500000000 --trim --split 500; https://github.com/rrwick/Filtlong), was performed with Unicycler v.0.4.51 (default settings). By combining short- and long-read data a complete hybrid 2.17Mb assembly was determined, which has been deposited, together with raw sequence data, in the European Nucleotide Archive (PRJEB26560).

Antimicrobial resistance determinants were identified from ONT and Illumina data as described previously using a combination of de novo assembly and mapping-based approaches.2

All previous N. gonorrhoeae Illumina whole-genome sequencing data in the NCBI Short Read Archive/European Nucleotide Archive, as of 29-March-2018, were downloaded. A k-mer based sequence comparison screen was undertaken using sourmash3 to identify closely-related genomes to our case, with a k-mer size of 31, 500 hashes, and scaled to 1000, after removing low abundance k-mers (copy number <10) with trim-low-abund.py v.2.1.1 and settings (-C 10 -M 2e9).45. 7812 sequences were available; 4 were excluded as they consisted entirely of low abundance k-mers. The 98 most closely related genomes to our case’s isolates’ sequences were compared in more detail by mapping reads using our hybrid assembly as a reference genome (identifying median(IQR) 90.3%(90.3-90.4%) of bases in the reference after quality filtering) and generating a recombination-
adjusted maximum-likelihood phylogeny as described previously. Additionally, all previously whole-genome sequenced ceftriaxone-resistant *N. gonorrhoeae* genomes as of 27-April-2018 were compared, using the NCCP11945 reference genome.

References


