Closed Genome Sequences of Clinical Neisseria gonorrhoeae Strains Obtained from Combined Oxford Nanopore and Illumina Sequencing

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ABSTRACT Neisseria gonorrhoeae is the etiological agent of gonorrhea, the second most common notifiable disease in the United States. Here, we used a hybrid approach combining Oxford Nanopore Technologies MinION and Illumina MiSeq sequencing data to obtain closed genome sequences of nine clinical N. gonorrhoeae isolates.

Neisseria gonorrhoeae is the causative agent of gonorrhea, the second most commonly reported notifiable disease in the United States (1). Gonorrhea poses a major public health threat, with emerging resistance to nearly all available antibiotics, including first-line dual therapy with azithromycin and ceftriaxone. Whole-genome sequencing (WGS) has become increasingly useful for tracking the spread and elucidating mechanisms of antimicrobial resistance (2–5). Prior to submission of our closed genomes to GenBank, there were 18 complete genome assemblies in the NCBI Reference Sequence Database (6). We report 9 new complete genomes spanning multiple sequence types.

N. gonorrhoeae isolates collected from symptomatic individuals for routine surveillance in Los Angeles, USA, were chosen for WGS. N. gonorrhoeae isolates were streaked onto Thayer-Martin chocolate agar plates (Hardy Diagnostics, Santa Maria, CA) and grown overnight at 37°C in a humidified 5% CO2 environment. For MinION sequencing, high-molecular-weight genomic DNA was prepared from bacteria scraped from Thayer-Martin agar using the Gentra Puregene Yeast/Bact. kit (Qiagen catalog number 158567) using manufacturer specifications. The Illumina sequencing libraries were prepared using the TruSeq library kit with bead-based size selection and were loaded onto an Illumina MiSeq flow cell. Sequencing was performed with a MiSeq reagent kit v3 in a 2 × 300-bp paired-end format. The MinION sequencing libraries were prepared using the rapid barcoding kit (catalog number SQK-RBK004) and loaded onto a single MinION R9.4 flow cell. Oxford Nanopore Technologies (ONT) reads were base-called with Albacore v2.0.2, generating 2.4 Gbp of data. Raw and trimmed Illumina reads were assessed using FastQC v0.11.7, and quality trimming was performed using Trimmomatic v0.32 (7) with the following settings: ILLUMINAACLIP, TruSeq3-PE-2.fa:2:30:10; LEADING, 3; TRAILING, 3; SLIDINGWINDOW, 4:24; and MINLEN, 60. ONT reads were demultiplexed and quality-trimmed using Porechop v0.2.3 (8) with default settings. A hybrid Illumina-ONT de novo assembly was performed using the Unicycler v0.4.7 pipeline (9) in
| Feature                                      | Data for strain:                                                                 |
|----------------------------------------------|----------------------------------------------------------------------------------|
| BioSample IDa                                | SAMN10395998, SAMN10395999, SAMN10396000, SAMN10396003, SAMN10395917, SAMN10395916, SAMN10395915, SAMN10395914, SAMN10395918 |
| Illumina read SRA accession no.              | SRR8457072, SRR8457069, SRR8457068, SRR8457071, SRR8457065, SRR8457064, SRR8457067, SRR8457066 |
| ONT read SRA accession no.                  | SRR8457081, SRR8457080, SRR8457079, SRR8457078, SRR8457076, SRR8457075, SRR8457074, SRR8457073 |
| Illumina read count (millions) (coverage []) | 2.31 (408), 2.81 (509), 2.39 (432), 2.06 (372), 1.61 (291), 1.97 (356), 2.15 (390), 2.59 (455), 0.41 (74) |
| ONT read count (thousands) (coverage [])    | 40.9 (66), 9.8 (19), 6.7 (38), 67.3 (134), 101.3 (206), 65.2 (116), 29.9 (66), 140.9 (293), 21.6 (51) |
| No. of circular contigs                      | 3, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2 |
| Plasmid length (bp)                          | 2,218,440, 2,228,346, 2,217,528, 2,232,677, 2,232,230, 2,218,771, 2,229,997, 2,217,835, 2,217,429 |
| GenBank accession no. (chromosome)           | CP034032, CP034030, CP034028, CP034026, CP034024, CP034022, CP034020, CP034018, CP034016 |
| GenBank accession no. (plasmids)             | CP034033, CP034031, CP034029, CP034027, CP034025, CP034023, CP034021, CP034019, CP034017 |
| GC content (%)                               | 52.39, 52.35, 52.45, 52.38, 52.38, 52.42, 52.35, 52.44, 52.45 |
| MLST                                         | 8122, 9363, 7363, 13149, 13149, 1901, 1901, 7363, 7363 |
| NG-MAST                                      | 292, 2992, 7374, 3506, 3506, 7631, 8476, 7574, 7574 |
| NG-STAR                                      | 299, 63, 1420 (novel), 1419 (novel), 1419 (novel), 550, 756, 540, 540 |

aID, identification number.
normal mode. Pilon v1.22 (10) was used iteratively (first using the flag --fix bases, then --fix all) to polish the assemblies with Illumina reads until no additional changes could be made. Detailed assembly statistics are provided in Table 1. The genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (6, 11). Multilocus sequence typing (MLST) and N. gonorrhoeae sequence typing for antimicrobial resistance (NG-STAR) were determined in silico using LOCUST (12) with default settings and are provided in Table 1. N. gonorrhoeae multiantigen sequence typing (NG-MAST) was determined in silico using NGMASTER (13) with default settings and is provided in Table 1. Novel NG-STAR profiles were confirmed via PCR (14, 15) and Sanger sequencing (Genewiz, South Plainfield, NJ) and uploaded to the NG-STAR database (16).

This study highlights the value of long-read sequencing in both detecting and closing plasmid sequences, as circular plasmids have been detected in only 22% of the 18 closed N. gonorrhoeae genomes, yet the cryptic plasmid is present in over 96% of isolates (17). We detected and completely assembled the N. gonorrhoeae cryptic plasmid in all nine isolates and detected and assembled the conjugal plasmid in strain FQ01 (Table 1).

Data availability. The complete genome sequences and the raw sequencing reads have been deposited in GenBank and the NCBI Sequence Read Archive (SRA), respectively, and are available under the accession numbers listed in Table 1.

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