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GENOME SEQUENCES

Complete Genome Sequence of *Neisseria gonorrhoeae* Multilocus Sequence Type ST7363 Isolated from Thailand

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ABSTRACT A *Neisseria gonorrhoeae* multilocus sequence type (MLST) ST7363 strain was isolated from a patient at the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, in 2010 and completely sequenced. This strain is susceptible to ceftriaxone and cefixime. A complete circular chromosome and circular plasmids were assembled from combined Oxford Nanopore Technologies (ONT) and Illumina sequencing.

Gonorrhea, caused by *Neisseria gonorrhoeae*, is among the most common sexually transmitted infections worldwide (1). Antimicrobial-resistant (AMR) *N. gonorrhoeae* is considered a high priority by the World Health Organization and an urgent threat by the U.S. Centers for Disease Control and Prevention (CDC) (2–5). Genomic analysis has been used to address the evolution and transmission of ceftriaxone-resistant genes (6, 7). We report here the complete genome sequence of a multilocus sequence type (MLST) ST7363 *N. gonorrhoeae* strain. This study was approved by the institutional review boards (IRBs) of the Faculty of Medicine Siriraj Hospital (certificate of approval number Si479/2015). The National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention (NCHHSTP), CDC, also reviewed and approved this study protocol and determined it to be research that did not involve identifiable human subjects, using unlinked or anonymous data or specimens, and therefore, CDC IRB approval was not required.

Based on our published data (8, 9), we selected the MLST ST7363 isolate from all frozen *N. gonorrhoeae* stock that was resistant to fluoroquinolone, penicillin, and tetracycline by disk diffusion (10) and harbored the bla_{TEM} gene for β-lactam resistance. The frozen isolates were cultured on chocolate agar in 5% CO_2 at 35°C and confirmed using Gram staining, oxidase and superoxol assays, and the API-NH (bioMérieux) biochemical test kit. An Etest (bioMérieux) was used to determine the MICs for ceftriaxone, cefixime, azithromycin, tetracycline, and gentamicin.

Genomic DNA was extracted from the colonies scraped from a chocolate agar plate using the Gentra Puregene yeast/bacteria kit (Qiagen). The extracted DNA was used for the Oxford Nanopore Technologies (ONT) and Illumina sequencing.

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TABLE 1 Assembly metrics and accession numbers of an MLST ST7363 *Neisseria gonorrhoeae* strain isolated from clinical specimens, Siriraj Hospital, Bangkok, Thailand, 2010

GenBank accession no.	Type of contig	Total length (bp)	Sequencing depth (×)		GC content (%)	No. of ORFs[a]
			ONT	Illumina		
CP045707.1	Circular chromosome	2,166,131	143	559	52.6	2,298
CP045708.1	Conjugative plasmid	42,907	658	1,912	47.6	51
CP045709.1	bla_{TEM} plasmid	5,154	34,644	10,363	38.4	5
CP045710.1	Cryptic plasmid	4,207	32,101	11,384	51.6	10

[a]ORFs, open reading frames.

The ONT library preparation followed the rapid barcoding sequencing protocol (SQK-RBK004; ONT), and sequencing was performed using an R9.4.1/FLO-MIN106 flow cell on a MinION device. We used Guppy v3.0.3 for base calling and demultiplexing of the reads. Quality control of the ONT reads followed the workflow from Jenjaroenpun et al. (11). The ONT adapters were trimmed using Porechop v0.2.3 (https://github.com/rrwick/Porechop). Reads with a mean quality score of 8 and a minimum read length of 1,000 bases were retained using NanoFilt v2.5.0 (12) for *de novo* assembly.

The Illumina library was prepared using a TruSeq DNA PCR-free library to generate 100-bp paired-end reads using the Illumina HiSeq platform. Quality control of the reads was performed using Fastp v0.19.5 (13). Hybrid assembly (ONT and Illumina data) was performed using Unicycler v0.4.4 (14) for genome error correction, circularization, and rotation. The genome sequence quality was determined using QUAST v5.0.2 (15) and submitted to the NCBI Prokaryotic Genome Annotation Pipeline v4.9 for genome annotation (16). Default parameters were used for all software.

The genome size was 2,218,399 bp. The assembly statistics and GenBank accession numbers are provided in Table 1. The N_{50} value/total read length (bp) of the ONT and Illumina reads were 4,248/182,670 and 100/13,878,684, respectively. A complete circular chromosome and plasmids, constructed using Unicycler software to check for overlapping sequences at the contig ends, demonstrated that the ST7363 isolate contained 3 circular plasmids, namely, conjugative, bla_{TEM}, and cryptic plasmids. *In silico* analysis confirmed sequence types of $bla_{TEM\text{-}135}$, MLST ST7363, and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) ST5225 (*por90*/*tbpB1106*). A novel sequence type, ST2209 (*penA2.002*, *mtrR19*, *porB11*, *ponA100*, *gyrA1*, *parC18*, and *23s100*), was uploaded to the NG-STAR database (17). The AMR determinants were defined using PubMLST (http://www.pubmlst.org/neisseria) (18). Type II nonmosaic *penA* possessed F504L, A511V, and A517G mutations, while *gyrA* had S91F and D95G mutations and *parC* had a D86N mutation. In contrast to the reported cephalosporin-resistant ST7363-*penA10.001/37.001/64.001*, our MLST ST7363-*penA2.002* was susceptible (Fig. 1) (19–21).

Data availability. The genome sequence has been submitted to GenBank under BioProject accession number PRJNA609415 and BioSample accession number SAMN13151449. The Illumina and ONT raw reads have been deposited in the SRA database under accession numbers SRR10362752 and SRR10388020.

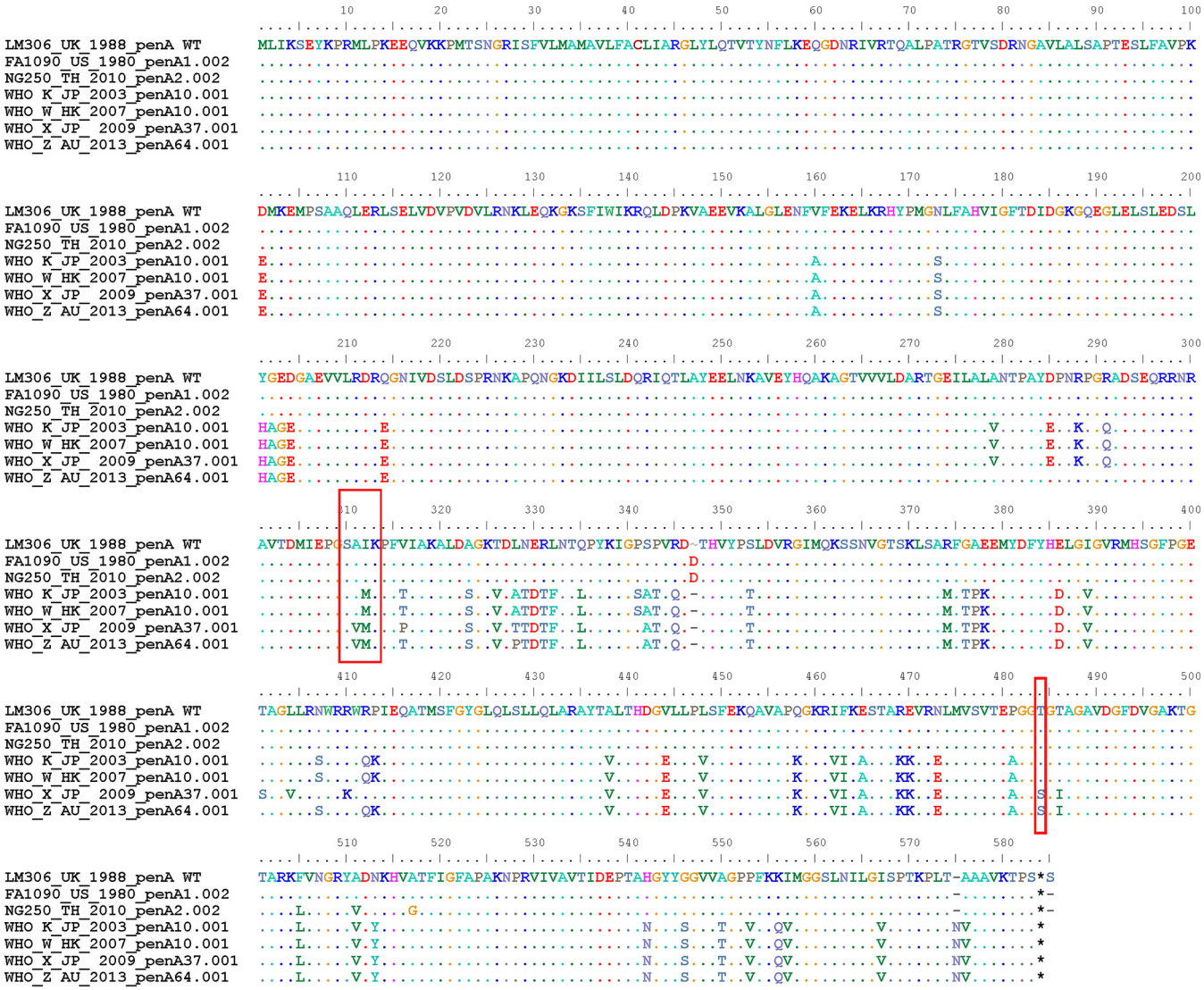

FIG 1 Multiple sequence alignment of penicillin-binding protein 2 (PBP2) coding sequences of the *penA* genes of ST7363 *Neisseria gonorrhoeae* from Thailand (NG250) with *penA2.002* (this study), compared with LM306 wild-type *penA*, the reference strain FA1090 (*penA1.002*), and four WHO reference strains, including WHO K (*penA37.001*), WHO W (*penA10.001*), WHO X (*penA37.001*), and WHO Z (*penA64.001*). All four of these WHO strains belong to ST7363 and cephalosporin-resistant *Neisseria gonorrhoeae* strains. The sequence titles of the strains are listed on the left in the following format: strain name_country source_year of isolation_*penA* type. The ruler with numbers on top of the sequences indicates the amino acid positions. The dot plots are a visual representation of the similarities between each sequence compared with the wild type in the first row. The A311V, I312M, and T483S mutations, which were previously reported in ceftriaxone-resistant strains and not found in our Thai susceptible strain, are indicated by the red rectangles. The MICs of strain NG250 for ceftriaxone and cefixime were 0.004 and 0.016 μg/ml, respectively.

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