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First Case of Ceftriaxone-Resistant Multidrug-Resistant *Neisseria gonorrhoeae* in Singapore

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KEYWORDS *Neisseria gonorrhoeae*, multidrug resistance

Amid the global crisis of increasing gonococcal antimicrobial resistance, we report the first case of ceftriaxone-resistant multidrug-resistant *Neisseria gonorrhoeae* in Singapore. Specimen 18DG342 was isolated from a throat swab taken from a female sex worker during a routine screening for sexually transmitted infections. The patient was empirically treated with intramuscular ceftriaxone 500 mg and azithromycin 1 g orally, based on local management guidelines (1). Repeated throat swab was culture negative for *N. gonorrhoeae* 1 week later. Two subsequent *N. gonorrhoeae* nucleic acid amplification tests (cobas 4800 CT/NG; Roche Diagnostics) were negative. It is possible that she was in contact with international clients as part of her sex work. Her travel history was unknown.

The isolate was cultured on GC-Lect agar (BD BBL) and was confirmed to be *N. gonorrhoeae* by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker), API NH (bioMérieux), and whole-genome sequencing. MIC values for ceftriaxone and 13 other antimicrobials were determined using Etests (bioMérieux) according to the manufacturer's instructions, and results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) breakpoints (2). Isolate 18DG342 was nonsusceptible to ceftriaxone (MIC, 1 mg/liter); resistant to cefixime (MIC, 4 mg/liter), penicillin (MIC, >32 mg/liter), and ciprofloxacin (MIC, >256 mg/liter); and of intermediate resistance to tetracycline (MIC, 1 mg/liter). It remained susceptible to azithromycin (MIC, 0.25 mg/liter) and spectinomycin (MIC, 16 mg/liter). Isolate 18DG342 was a β-lactamase-producing strain based on the positive Cefinase paper disc (BD BBL) test. Based on Australian Gonococcal Surveillance Program (AGSP) (http://cdstest.net/manual/neisseria-gonorrhoeae/) interpretative criteria (3–5), 18DG342 was less sensitive to gentamicin (MIC, 8 mg/liter).

We performed molecular typing *in silico* using whole-genome sequence data (BioProject accession no. PRJNA508549). We sequenced the isolate with the Illumina MiSeq platform (Illumina) and used the genomic quality, assembly, and phylogenetic analysis pipeline as described previously (6). Sequence data were submitted to the *Neisseria* multilocus sequence typing (MLST) website (https://pubmlst.org/neisseria/) (7) and were assigned the novel sequence type ST13871. While most previously reported ceftriaxone-resistant *N. gonorrhoeae* isolates belong to MLST ST1903 (*fumC* allele 157), 18DG342 differed in the *fumC* locus (*fumC* allele 987). The *N. gonorrhoeae* multiantigen sequence type (NG-MAST) (http://www.ng-mast.net/) (8) was ST1086 (*porB* allele number 581 and *tbpB* allele number 21). *N. gonorrhoeae* sequence typing for the antimicrobial resistance (NG-STAR) profile was 233 (https://ngstar.canada.ca/pages/welcome?lang=en) (9). NG-STAR profile 233 contains a mosaic *penA*-60.001 allele, an *mtrR*-35A deletion, and *porB* G120K, *ponA* L421P, *gyrA* S91F/D95A, and *parC* S87R mutations. 23S rRNA mutations were not detected. $bla_{TEM-135}$ was detected in the whole-genome

Citation Ko KKK, Chio MTW, Goh SS, Tan AL, Koh TH, Abdul Rahman NB. 2019. First case of ceftriaxone-resistant multidrug-resistant *Neisseria gonorrhoeae* in Singapore. Antimicrob Agents Chemother 63:e02624-18. https://doi.org/10.1128/AAC.02624-18.

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Accepted manuscript posted online 11 March 2019
Published 25 April 2019

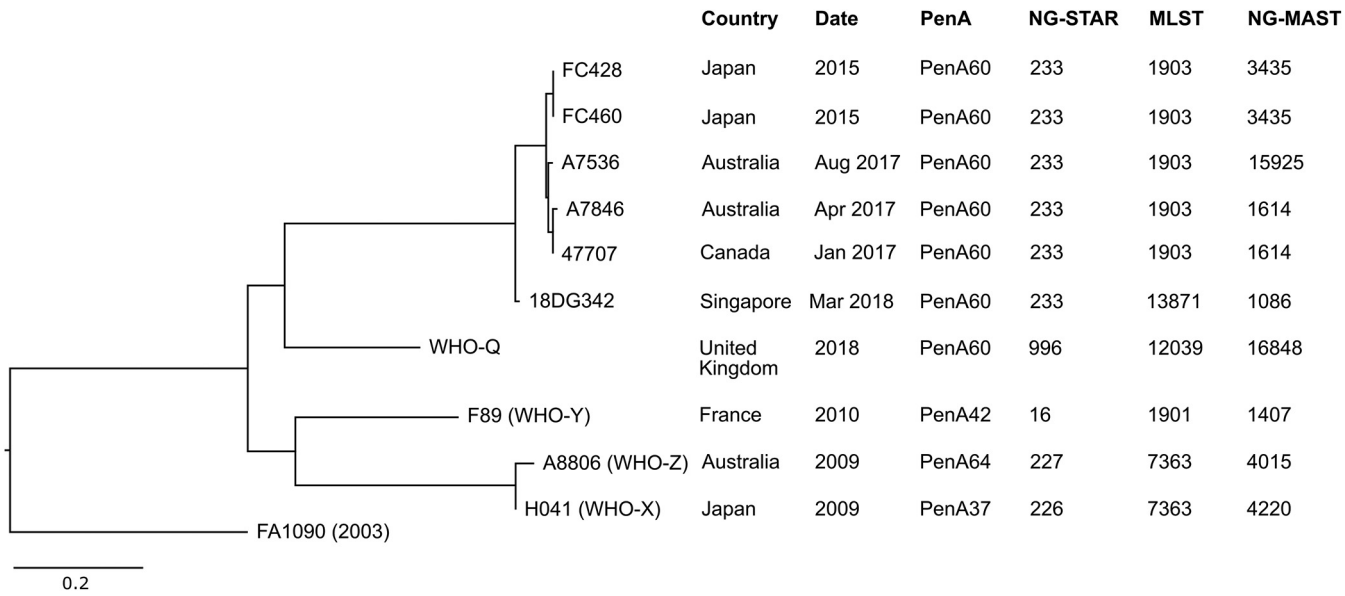

FIG 1 Core genome single nucleotide variation (SNV) phylogenetic tree of the ceftriaxone-resistant multidrug-resistant *Neisseria gonorrhoeae* isolate 18DG342 and a panel of previously reported ceftriaxone-resistant *N. gonorrhoeae* isolates (10–15). The complete genome of *N. gonorrhoeae* FA1090 (GenBank accession no. NC_002946.2) was included as the reference sequence. Scale bar indicates nucleotide substitution per site. PenA, penicillin-binding protein 2; NG-STAR, *Neisseria gonorrhoeae* sequence type for antimicrobial resistance; MLST, multilocus sequence type; NG-MAST, *Neisseria gonorrhoeae* multiantigen sequence type.

sequence data. The molecular antimicrobial resistance profile corresponds to the MICs determined phenotypically.

The 18DG342 genome sequence was compared with those of previously genome-sequenced ceftriaxone-resistant isolates FC428 (10), F460 (10), 47707 (11), A7536 (12), A7846 (12), H041 (13), A8806 (13), F89 (13), and WHO-Q (G97687/G7944) (14). A single nucleotide variation (SNV) phylogenetic tree was created by mapping reads to the reference sequence FA1090 (GenBank accession no. NC_002946.2) (Fig. 1). By core genome SNV analysis (12, 15), 18DG342 was distinct from the previously described H041 (WHO-X), F89 (WHO-Y), and A8806 (WHO-Z) ceftriaxone resistance strains and was more closely related to the internationally spreading Japanese FC428 clone. Isolate 18DG342 was most closely related to the MLST ST1903 strains (FC428, FC460, A7536, A7846, and 47707), with a difference of 35 to 39 SNVs between the core genomes of 18DG342 and the ST1903 strains included in this analysis.

Situated in Southeast Asia with a large transient population of foreign visitors, the importation and dissemination of antimicrobial resistance is a constant public health threat to Singapore. Ceftriaxone-resistant multidrug-resistant *N. gonorrhoeae* undermines the effectiveness of currently recommended first-line dual therapy. Enhanced surveillance of antimicrobial susceptibilities is necessary to detect and monitor the resistance trends so as to safeguard the effectiveness of the remaining therapeutic options. The emergence of ceftriaxone-resistant gonococcal infection had been anticipated (1), and effective control measures (e.g., mandatory regular screening of sex workers, safe sex practice advice to sex workers) are in place to safeguard public health (https://sso.agc.gov.sg/Act/IDA1976). The detection of this gonococcal isolate serves as a stark reminder of the continual spread of multidrug-resistant *N. gonorrhoeae* globally and calls for even greater vigilance to limit the potential of dissemination.

Accession number(s). Raw sequencing reads have been deposited at GenBank under BioProject accession no. PRJNA508549.

ACKNOWLEDGMENTS

The sequencing was funded by a SingHealth Pathology Academic Clinical Program Clinical Innovation Support Program grant.

We declare no conflicts of interest.

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