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

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Amid the global crisis of increasing gonococcal antimicrobial resistance, we report the first case of ceftriaxone-resistant multidrug-resistant *Neisseria gonorrhoeae* in Singapore. 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 500mg and azithromycin 1g orally, based on local management guidelines (1). Repeated throat swab was culture negative for *N. gonorrhoeae* one 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. Minimum inhibitory concentration (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 the Clinical and Laboratory Standards Institute (CLSI) breakpoints (2). 18DG342 was non-susceptible to ceftriaxone (MIC 1mg/L), resistant to cefixime (MIC 4mg/L), penicillin (MIC >32mg/L) and ciprofloxacin (MIC >256mg/L) and of intermediate resistance to tetracycline (MIC 1mg/L). It remained susceptible to azithromycin (MIC 0.25mg/L) and spectinomycin (MIC 16mg/L). 18DG342 was a β-lactamase producing strain based on the positive cefinase paper disc (BD BBL™) test. Based on Australian Gonococcal Surveillance...
Program (AGSP) interpretative criteria (3,4,5), 18DG342 was less sensitive to gentamicin (MIC 8mg/L).

We performed molecular typing in silico using whole genome sequence data (BioProject PRJNA508549). We sequenced the isolate with the Illumina MiSeq platform (Illumina) and used genomic quality, assembly and phylogenetic analysis pipeline as described previously (6). Sequence data was submitted to the Neisseria Multi Locus Sequence Typing (MLST) website (https://pubmlst.org/neisseria/) (7), and was 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). N. gonorrhoeae multiantigen sequence type (NG-MAST) (http://www.ng-mast.net/) (8) was ST1086 (porB allele number 581 and tbpB allele number 21). The N. gonorrhoeae sequence typing for antimicrobial resistance (NG-STAR) profile was 233 (https://ngstar.canada.ca/welcome/home) (9). NG-STAR profile 233 contains a mosaic penA-60.001 allele, mtrR-35A deletion, porB G120K, porA L421P, gyrA S91F/D95A and parC S87R mutations. 23S rRNA mutations were not detected. blaTEM-135 was detected in the whole genome sequence data. The molecular antimicrobial resistance profile corresponds to the MICs determined phenotypically.

The 18DG342 genome sequence was compared with 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) (Figure 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. 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 \textit{N. gonorrhoeae} undermines the effectiveness of the 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 \cite{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 \textit{N. gonorrhoeae} globally and calls for even greater vigilance to limit the potential of dissemination.

**Figure legend:**
Core genome single nucleotide variation (SNV) phylogenetic tree of the ceftriaxone-resistant multidrug-resistant \textit{Neisseria gonorrhoeae} isolate 18DG342 and a panel of previously reported ceftriaxone-resistant \textit{N. gonorrhoeae} isolates \cite{10,11,12,13,14}. The complete genome of \textit{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, \textit{Neisseria gonorrhoeae} sequence type for antimicrobial resistance; MLST, multilocus sequence type; NG-MAST, \textit{Neisseria gonorrhoeae} multiantigen sequence type.

**Accession numbers:**
Raw sequencing reads have been deposited at GenBank under Bioproject PRJNA508549.

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We declare no conflicts of interest.
References:


3. Clinical and Laboratory Standards Institute, Wayne, PA.


http://cdstest.net/


Figure 1.

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