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A Case of Decreased Susceptibility to Ceftriaxone in *Neisseria gonorrhoeae* in the Absence of a Mosaic Penicillin-Binding Protein 2 (*penA*) Allele

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Abstract

We report a case of *Neisseria gonorrhoeae* with a non-mosaic *penA* allele that exhibited decreased susceptibility to extended-spectrum cephalosporins, including a ceftriaxone minimum inhibitory concentration of 0.5 μg/mL. An analysis of resistance determinants suggested that the observed phenotype might have resulted from the combined effects of mutations in multiple genes.

Neisseria gonorrhoeae is the causative agent of the sexually transmitted disease (STD) gonorrhea, which is the second most commonly reported notifiable disease in the United States.[1] Gonococcal infections are significant concerns due to the emergence of resistance to multiple antibiotics, which has been facilitated by both plasmid- and chromosome-mediated mechanisms.[2] As a result of this acquired resistance, particularly to extended-spectrum cephalosporins (ESCs), the Centers for Disease Control and Prevention currently recommends dual therapy with ceftriaxone plus azithromycin as treatment for uncomplicated gonorrhea,[3] and the treatment has been effective in the United States. Between 1987 and 2015, 5 isolates exhibiting decreased ceftriaxone susceptibility (minimum inhibitory concentration [MIC] = 0.5 μg/mL) were reported in the United States via the Gonococcal Isolate Surveillance Project (GISP) (https://www.cdc.gov/std/gisp/),[1] including the isolate described in this case report.

In February 2012, a 32-year-old African-American male with symptoms of urethritis visited an STD clinic in Oklahoma City, Oklahoma that participated in GISP at that time. He reported recent sexual contact with a single female partner and denied prior gonococcal infection, current human immunodeficiency virus infection, sex with men, involvement with

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the commercial sex trade, or recent travel. However, his female partner reported that she recently had sexual contact with a partner in Chicago, Illinois.

A urethral culture was obtained for inclusion in the GISP collection, and it tested positive for *N. gonorrhoeae*. The patient was treated with 250-mg ceftriaxone as a single intramuscular injection, and he was prescribed 100-mg doxycycline to be taken orally twice daily for 1 week, based on the recommended treatment guidelines at that time. The asymptomatic female partner was located, and her urine sample tested positive for *N. gonorrhoeae* using a gonococcal-specific nucleic acid amplification test. The sample was tested using the BD Viper System (BD, New Jersey) in extracted mode using strand displacement amplification. A throat culture obtained from the female partner was negative for *N. gonorrhoeae*. She was also treated with a combination of ceftriaxone and doxycycline.

The urethral culture from the patient was subjected to antimicrobial susceptibility testing at a GISP reference laboratory at the Texas Department of State Health Services to determine the MICs for target antibiotics. Agar dilution testing revealed decreased susceptibility to the following ESCs: ceftriaxone (MIC = 0.5 μg/mL), cefixime (MIC = 1.0 μg/mL), and cefpodoxime (MIC = 2.0 μg/mL). The isolate was also resistant to penicillin (MIC = 2.0 μg/mL), and the tetracycline MIC value (MIC = 1.0 μg/mL) was one dilution from the Clinical and Laboratory Standards Institute breakpoint of 2 μg/mL for resistance. The isolate exhibited susceptibility to both azithromycin (MIC = 0.5 μg/mL) and ciprofloxacin (MIC = 0.015 μg/mL). These results were confirmed via agar dilution at the Centers for Disease Control and Prevention. The Oklahoma City-County Health Department STD program was notified of the results, and a health alert was issued to local healthcare providers on June 21, 2012. Numerous unsuccessful attempts were made by the STD program to locate the patient and his primary partner after the initial visit.

To further characterize the isolate (hereafter referred to as isolate 12CFX_T_009), whole-genome sequencing was used to identify resistance determinants associated with decreased susceptibility to cefixime and other extended-spectrum cephalosporins. Whole-genome sequencing was conducted using both short-read (Illumina HiSeq 2500 platform; European Nucleotide Archive accession number SAMEA3165247) and long-read (PacBio RSII platform) techniques. Regarding short-read data, de novo assembly and variant calling were conducted using SPAdes[4] and CLC Genomics Workbench 9.0 software (www.qiagenbioinformatics.com), respectively, and the complete genome of gonococcal strain FA19 (Genbank accession no. CP012026) was used as a reference for variant calling. De novo assembly of long-read sequence data was conducted using the hierarchical genome assembly process workflow, which included consensus polishing using Quiver.[5] To avoid variants due to sequencing errors, the short-read data were mapped to the assembled long-read data (assembled as a single contig), and variant calling was conducted using CLC Genomics Workbench 9.0 software.

The following known gonococcal resistance determinants were examined to identify mutations potentially associated with reduced susceptibility to β-lactam antibiotics and ESCs: penicillin-binding protein 2 (*penA*), penicillin-binding protein 1 (*ponA*), multiple transferable resistance repressor (*mtrR*), porin B (*porB*), and type IV pilus biogenesis and

competence protein PilQ (*pilQ*).[6,7] In addition, both major pilin subunit (*pilE*) and type IV pilus tip-located adhesion PilC (*pilC1* and *pilC2*) genes were examined because the products of these genes interact with the *pilQ* product during pilus formation.[8,9]

The results of the comparison of the short- and long-read sequence data indicated an error rate of 0.01% between the 2 methods, and no discrepancies were found between sequences of the target genes. The results of *N. gonorrhoeae* multiantigen sequence typing and multilocus sequence typing analyses identified the strain as ST14647 and ST1893, respectively. Variation was detected in each of the target resistance genes (Table 1). Mosaic *penA* alleles are strongly associated with reduced susceptibility to cefixime and ceftriaxone.[10] However, the results indicated that the *penA* allele for isolate 12CFX_T_009 was a non-mosaic *penA* IX allele, which has not been associated with reduced susceptibility to ESCs. Regarding *ponA*, a single mutation (421P) associated with reduced susceptibility to cephalosporins was identified.[6] However, this particular *ponA* mutation has not been linked to ceftriaxone MIC values greater than 0.125 μg/mL.[11] Mutations in the *mtrR* promoter (a single adenine deletion) and the *mtrR* coding region (45D) can lead to increased expression of the MtrCDE efflux pump, which could lead to reduced susceptibility to ESCs.[6,12] Although the 45D mutation was detected (in the absence of the promoter mutation), it has not been associated with ceftriaxone MIC values comparable to that observed in this case. *porB* exhibited extensive variability, but only mutations at residues 120 and 121 in this gene have been associated with resistance to β-lactam antibiotics.[13] However, the *porB* mutations identified in this case (120N and 121A) have not previously been associated with resistance to β-lactam antibiotics. Regarding *pilQ*, the 666K mutation is associated with resistance to penicillin based on in vitro experimentation,[14] but this mutation has not been identified in clinical samples.[15] The 666K mutation was not found is this case, and the 3 identified mutations have not been associated with resistance to β-lactam antibiotics in clinical isolates. The greatest variability was observed in *pilE, pilC1*, and *pilC2*, which contained 14, 198, and 366 missense mutations, respectively. Although specific mutations in these genes are not associated with resistance, the products of these genes interact with the *pilQ* product during pilin synthesis; therefore, drastic mutations (e.g., nonsense mutations resulting in nonfunctional or truncated products) could affect the latter. However, no early stop codons were identified during examinations of the transcribed amino acid sequences of these 3 genes.

Although a strong association between mosaic *penA* alleles and increased resistance to ESCs in *N. gonorrhoeae* has been established,[10] reduced susceptibility to ESCs has also been detected in isolates containing non-mosaic penA alleles.[7,16,17] Although the isolate described in this case exhibited a non-mosaic *penA* allele in conjunction with several mutations in other resistance loci (*ponA, mtrR, porB*, and *pilQ*), none of the identified mutations were previously individually associated with the observed elevated ceftriaxone MIC levels (0.5 μg/mL). Therefore, the results of this analysis suggest that in the absence of major mutations, the observed reduced susceptibility might be due to a multilocus phenomenon resulting from the combined effects of mutations at multiple targets or via unknown compensatory changes at secondary loci.[18] Furthermore, these results suggest that molecular tests that aim to identify reduced susceptibility to ESCs in *N. gonorrhoeae* will have to take multiple mutations and mutational combinations into account to obtain accurate

diagnoses. Future analyses of this and similar isolates warrant the investigation of these mechanisms, which could drastically impact the extent of antimicrobial resistance in *N. gonorrhoeae*.

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TABLE 1.

Target Resistance Genes and Associated Mutations

Gene	Mutations*
penA	ins346D, 505L, 511V, 517G, 542H, and 552L; non-mosaic IX
ponA	421P
mtrR	45D
porB	80 missense including 120N and 121A
pilQ	341N, 523G, and 648N
pilC1	198 missense
pilC2	366 missense
pilE	14 missense

* Insertions and missense mutations relative to FA19 nucleotide sequences.