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First Case of High-Level Azithromycin-Resistant *Neisseria gonorrhoeae* in North Carolina

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Abstract

We report on the first high-level azithromycin-resistant *Neisseria gonorrhoeae* isolate (minimum inhibitory concentration, ≥256 μg/mL) in North Carolina isolated from a pharyngeal swab of a 33-year-old HIV-negative man who has sex with men. In addition, the isolate was found to be susceptible to cefixime, ceftriaxone, and penicillin and resistant to tetracycline. By whole-genome sequencing, the strain was assigned as MLST ST9363, NG-MAST ST5035, and a novel NG-STAR sequence type, ST1993.

Sexually transmitted infections are one of the major public health concerns around the world. Gonorrhea, which is caused by the bacterium *Neisseria gonorrhoeae*, is the second most common bacterial sexually transmitted infection. In 2018, there were 231 cases of reported gonorrhea per 100,000 populations in North Carolina (NC), higher than the national rate of 179.1 cases per 100,000 populations.[1]

N. gonorrhoeae has progressively developed resistance to the antimicrobial agents recommended for treatment of infections, including sulfonamides, penicillins, tetracyclines, fluoroquinolones, macrolides, and cephalosporins.[2] In 2001, we saw the emergence of a strain of *N. gonorrhoeae* out of Argentina with high-level azithromycin resistance,[3] which subsequently emerged in different countries around the world.[4]

We describe here an *N. gonorrhoeae* isolate with high-level azithromycin resistance but susceptible to cefixime and ceftriaxone. This organism was isolated from the culture of a pharyngeal swab of a 33-year-old HIV-negative man who has sex with men and a patient at the Guilford County Health Department. The patient reported a 1-month history of yellow urethral discharge and penile itching. He reported unprotected, insertive anal and

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oral sex with 2 male partners in the 60 days before his visit and denied having sex with an intravenous drug user or recent travel history outside the United States in the past 3 months. The patient was evaluated for genital discharge and tested by Gram stain, culture, and *N. gonorrhoeae* and *Chlamydia trachomatis* nucleic acid amplification test (NAAT; Panther System; Hologic, San Diego, CA). In addition, a pharyngeal sample was collected for culture and NAAT testing. The Gram stain performed on site identified less than 2 white blood cells per high-power field and no gram-negative intra-cellular diplococci. The patient was discharged from the clinic with the plan to follow up should NAAT testing results return positive.

On the pharyngeal sample, suspected colonies were observed 5 days after collection. The colonies were identified as *N. gonorrhoeae* by Gram stain, oxidase reaction, BactiCard Neisseria (Remel; ThermoFisher Scientific, Lenexa, KS), and API NH identification system (BioMerieux, Durham, NC). The pharyngeal sample was also positive for *N. gonorrhoeae* by NAAT. The urethral sample was negative for *N. gonorrhoeae* by culture and negative for *C. trachomatis* and *N. gonorrhoeae* by NAAT. With the support of the Centers for Disease Control and Prevention's (CDC's) Strengthening the US Response to Resistant Gonorrhea, antimicrobial susceptibility testing for cefixime, ceftriaxone, and azithromycin was determined by ETEST (bioMerieux, Durham, NC), and penicillin, tetracycline, and gentamicin were determined by agar dilution performed at the Tennessee Department of Health. Results were interpreted in accordance with the Clinical and Laboratory Standards Institute breakpoints.[5] The isolate was nonsusceptible to azithromycin (minimum inhibitory concentration [MIC], ≥256 μg/mL). It was susceptible to cefixime (MIC ≤0.016 μg/mL), ceftriaxone (MIC ≤0.016 μg/mL), and penicillin (MIC, 1 μg/mL), but resistant to tetracycline (MIC, 4 μg/mL). Treatment with ceftriaxone 250 mg intramuscularly once and azithromycin 1 g by mouth once was performed per CDC recommendations.[6] Patient symptoms were resolved, and results from follow-up NAAT testing and cultures were negative at 2 and 8 weeks after treatment. No contacts were identified or tested.

This isolate was sequenced at the Tennessee Department of Health, which is one of the *N. gonorrhoeae* Antibiotic Resistance Laboratory Network regional laboratories. The sequence data were transferred to CDC for additional quality control and genomic analysis using CDC's *N. gonorrhoeae* AR Profiler and Typing Tool. Molecular characterization was performed at the CDC using multilocus sequence typing (MLST), *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), and *N. gonorrhoeae* sequence typing for antimicrobial resistance (NG-STAR) on the whole-genome sequence generated with the MiSeq platform (Illumina Denmark ApS, Copenhagen, Denmark). Whole-genome sequence quality assessment and read assembly were performed as described.[7] Sequence data were submitted to the *Neisseria* MLSTwebsite (https://pubmlst.org/neisseria/),[8] and the strain was found to be MLST ST9363. Analysis of the isolate by NG-MAST (https://www.ng-mast.net/) designated the NC strain as NG-MAST ST5035. Analysis by NG-STAR identified a novel NG-STAR sequence type, ST1993. The allele profile included *penA*-2.001 type II nonmosaic allele, *mtrR*-39 mosaic-like allele, *porB*-11 G120K and A121N, *ponA*-100 wild type, *gyrA*-100 wild type, *parC*-100 wild type, and 23S-1 A2059G.

The NG-MAST typing scheme examines variable internal fragments of 2 genes, *porB (*490 bp) and *tbpB* (390 bp). *N. gonorrhoeae* multiantigen sequence typing can be used for identifying clusters and is a convenient molecular typing method for *N. gonorrhoeae*, which has high discriminatory power and high reproducibility for assessing molecular relatedness.[9] After sequence typing by NG-MAST, the NC strain was assigned NG-MAST ST5035 (*porB* allele, 1914; *tbpB* allele, 29). By NG-MAST typing, the NC strain described here is unrelated to other azithromycin-resistant isolates reported from other countries such as Scotland (ST470, ST649), Ireland (ST649, ST3311), Italy (ST2142), Sweden (ST285, ST8727), Argentina (ST696), England (ST9768), and Canada (ST1948).[4] In the United States, the first *N. gonorrhoeae* with high-level resistance to azithromycin was reported in Hawaii. That strain contained the 23S rRNA mutation A2059G and was determined to be NG-MAST ST649 (*porB* allele, 442; *tbpB* allele, 29).[10] The NG-MAST ST10844 strain with high-level azithromycin resistance detected in California also possessed *tbpB* allele 29 and had a *porB* allele 6354 that was closely related to 442 with 1-bp difference.[11] Although, the NC strain had an identical *tbpB* allele with ST649 and ST10844, it had a *porB* allele that was not closely related to *porB* 442 and 6354 with a total of 29- and 38-bp difference, respectively (Fig. 1). The raw sequence data for the NC strain (GCWGS_4011) were deposited in GenBank (accession no. SRR9878077). The azithromycin-resistant *N. gonorrhoeae* ST649 and ST10844 strains isolated in Hawaii and California, respectively, were detected in young, heterosexual adults. The NC strain was isolated from a man who has sex with men, a population in which emerging resistance to *N. gonorrhoeae* has been reported.[13] In 2016, the Hawaii Department of Health reported 8 high-level azithromycin-resistant strains isolated from 7 patients. The results of the NG-MAST typing for those isolates revealed that all 8 shared 1 novel ST14121 profile (*porB* allele, 485; *tbpB* allele, 110), suggesting the circulation of a single strain within the population. However, the azithromycin-resistant isolate ST649 isolated in Hawaii in 2011 was placed in a distinct clade on the phylogenetic tree compared with these 8 isolates isolated more recently.[14]

Thomas et al[7] sequenced genomes of 649 isolates collected through the Gonococcal Isolate Surveillance Project in the United States and found that MLST ST9363 and NG-MAST ST3995 were most prevalent in elevated azithromycin MIC strains. However, the predominant MIC range for azithromycin of these MLST ST9363 isolates was 2 to 4 μg/mL. They also identified the 23S rRNA A2059G mutation in 7 azithromycin high-level resistance strains (MICs ≥256 μg/mL) represented by MLST ST7822, which is a different MLST type from the NC strain.

The NG-STAR typing scheme allows for the analysis of the genes associated with antimicrobial resistance and is useful for the identification of clusters with similar antimicrobial resistance profiles as well as detection of novel alleles associated with resistance. It is known that 23S rRNA point mutations, including the A2059G, are associated with high-level azithromycin resistance when present in 3 or 4 of the 4 alleles of the 23S rRNA gene.[4] The A2059G mutation was well documented in some bacteria such as *Escherichia coli*, *Helicobacter pylori*, *Streptococcus pneumoniae*, and *Mycoplasma pneumonia*, but it was reported for the first time in a *N. gonorrhoeae* strain isolated in Argentina in 2009.[15] The strain presented here contains both the A2059G mutation in all 4 alleles of the 23S rRNA *rrl* gene and the G120K and A121N mutations in the *porB* coding

region. The A2059G mutation detected in the NC strain was the same as other high-level azithromycin-resistant gonococcal strains that have been reported in other countries and the United States.[4,15,16]

The NC strain described here shares both the 23S rRNA A2059G mutation and the mosaic-like mtrR locus, which is very similar to the strains described by Pham et al.[16] However, a much more expansive phylogenetic analysis of strains both in NC and outside NC would need to be done to understand whether the NC strain arose locally through genetic mutation or as a part of an existing clone imported to NC. This case highlights the importance of local surveillance for monitoring trends in antimicrobial resistance of *N. gonorrhoeae*.

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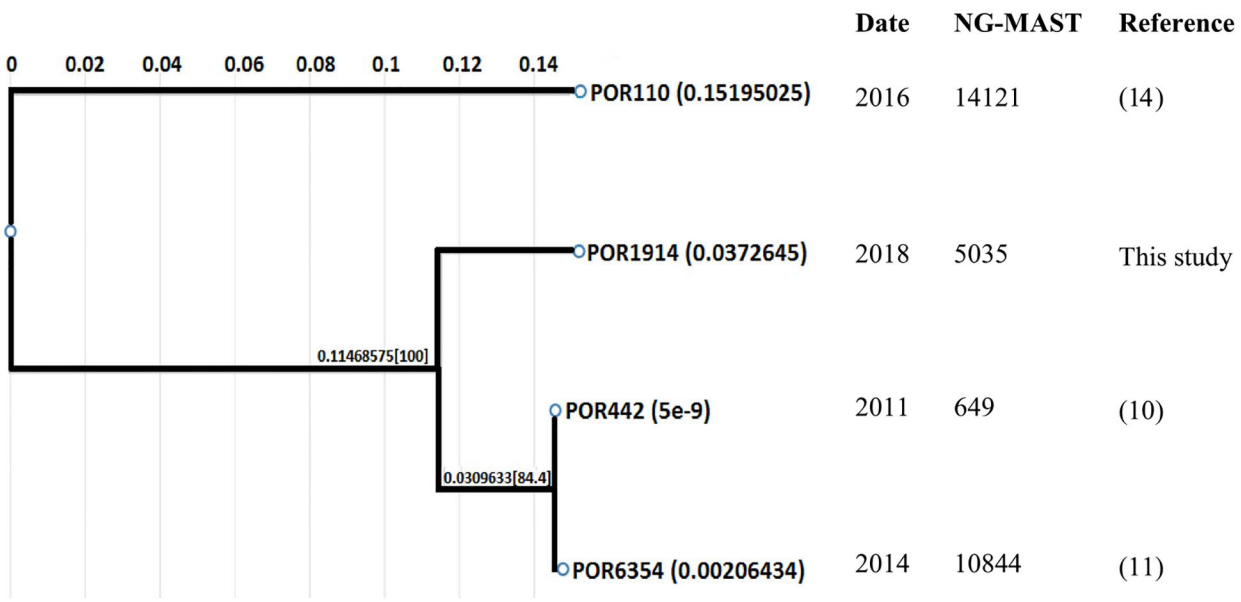

Figure 1.

porB gene single-nucleotide polymorphism phylogenetic tree of the high-level azithromycin-resistant NC-1263 strain and a panel of previously reported high-level azithromycin-resistant *N. gonorrhoeae* isolates in the United States. Scale bar indicates nucleotide substitutions per site. POR110 was used as a reference. The tree was constructed using fasttree with slow nearest-neighbor interchanges and MLACC = 3 (to make the maximum-likelihood nearest-neighbor interchanges more exhaustive). NG-MAST indicates *N. gonorrhoeae* multiantigen sequence typing.[12]