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BRIEF REPORT

Neisseria gonorrhoeae With High-Level Resistance to Azithromycin: Case Report of the First Isolate Identified in the United States

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We report on the first *Neisseria gonorrhoeae* isolate in the United States identified with high-level resistance to azithromycin. This report discusses the epidemiologic case investigation, the molecular studies of resistance-associated mutations and *N. gonorrhoeae* multiantigen sequence typing, and challenges posed by emerging gonococcal antimicrobial resistance.

On 28 January 2011, an asymptomatic 21-year-old white woman attending college in Hawaii presented to a clinic in Honolulu participating in the Hawaii Department of Health (HDOH) Gonorrhea Culture Screening Program. She sought care because 1 day earlier, her male sex partner had been seen for symptoms of dysuria and urethral discharge at a private clinic in Honolulu. He was presumptively treated with 250 mg of ceftriaxone intramuscularly and 1.0 g of azithromycin orally. A urethral specimen from him tested positive for *Neisseria gonorrhoeae* and negative for *Chlamydia trachomatis* using a nucleic acid amplification test (NAAT). She was treated with 250 mg of ceftriaxone intramuscularly and 1.0 g of azithromycin orally on the day of her clinic visit. An endocervical specimen from her was later positive for *N. gonorrhoeae* by culture; a second specimen was negative for *C. trachomatis* by NAAT.

The gonococcal isolate was subsequently tested on 2 May 2011 for antimicrobial resistance using Etest (BioMérieux Clinical Diagnostics, Marcy l'Etoile, France), which demonstrated a minimum inhibitory concentration (MIC) of azithromycin >256 μg/mL. The isolate was sent to the Centers for Disease Control and Prevention's (CDC) Gonococcal Isolate Surveillance Project (GISP) Regional Laboratory (University of Washington, Seattle), where testing by reference agar dilution method [1] revealed an MIC of azithromycin >512 μg/mL. The isolate was resistant to tetracycline (MIC = 2.0 μg/mL) but susceptible to cefixime (MIC = 0.125 μg/mL), ceftriaxone (MIC = 0.03 μg/mL), and cefpodoxime (MIC = 0.25 μg/mL). Although cefixime susceptible, the MIC was 1 dilution from the Clinical and Laboratory Standards Institute (CLSI) breakpoint of 0.25 μg/mL for susceptibility. A field investigation by HDOH was initiated, and molecular characterization of the isolate was undertaken.

RESULTS

Field Investigation

The patient and her partner were interviewed 5 May 2011 at the HDOH sexually transmitted disease (STD) clinic. She denied previous STD-related symptoms and last had negative screening tests for *N. gonorrhoeae* and *C. trachomatis* on 25 May 2010. The 2 claimed mutual monogamy since early December 2010. She reported that sexual activity with a different partner last occurred in September 2010. The patient traveled home to California from 15 December 2010 through 10 January 2011; she and her partner denied sexual contact during this period. Both also denied antibiotic use 30 days prior to diagnosis. Her male partner reported that sexual activity with a different partner last occurred with a female in October 2010. This female was promptly traced to San Francisco, where she was tested for *N. gonorrhoeae* and *C. trachomatis* by NAAT and found to be uninfected.

A medical alert was developed by HDOH and distributed to all licensed physicians in Hawaii on 25 May 2011 [2] to coincide with the 24 May 2011 release of a "Dear Colleague" letter from CDC, announcing the azithromycin-resistant isolate [3].

Molecular Studies

The Hawaii gonococcal strain H11S8 was characterized by *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) [4], and screened for azithromycin resistance-associated mutations in the peptidyltransferase region of domain V of the 23S rRNA

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gene using a previously described 2-step polymerase chain reaction (PCR) method and sequencing [5]. PCR assays were also performed to determine the carriage of efflux pump *mef*(A) gene and methylase-encoding *erm*(A), *erm*(B), and *erm*(C) genes, using previously described primers and cycling parameters [6, 7]. The *mtrR* promoter region was also sequenced and screened for a mutation described previously to be associated with upregulation of the MtrCDE efflux pump [8].

Strain H11S8 was sequence type (ST) 649 by NG-MAST. It contained mutation A2059G (*Escherichia coli* numbering) in all 4 23S rRNA gene alleles but did not have the C2611T mutation, which has been reported previously in the 23S rRNA gene of moderately azithromycin-resistant *N. gonorrhoeae* isolates from Canada [5]. Strain H11S8 was negative by PCR for *erm*(A), *erm*(B), *erm*(C), and *mef*(A) genes and contained no mutation in the *mtrR* promoter region.

DISCUSSION

This is the first report of a gonococcal isolate with high-level resistance to azithromycin identified in the United States. The case investigation did not discern whether the male partner's infection had been acquired in Hawaii while his girlfriend was in California, and transmitted to her after her return; or whether she had acquired gonorrhea in California and transmitted to him upon her return. Eight isolates from Hawaii with reduced susceptibility to azithromycin (MICs = 8–16 μg/mL) were previously identified by GISP: 1 in 1999, 1 in 2006, 3 in 2008, 1 in 2009, and 2 in 2010 (CDC, unpublished data). Since 2004, GISP has identified 55 isolates from other US states with azithromycin MICs of 8–16 μg/mL; 15 were identified in 2010 (CDC, unpublished data), including 4 from San Diego, California [9]. Gonococcal isolates with high-level resistance to azithromycin (MICs >256 μg/mL) have previously been identified from Argentina in 2001 [10], Scotland in 2004 [11], England and Wales in 2007 [12], and Ireland in 2008 [13]. Isolates with MICs ≥128 μg/mL were also identified from Italy in 2007 [14].

Gonococcal strain H11S8 from the index patient was ST649, which has been previously reported among young heterosexual adults in Scotland [11], England, and Wales [12]. Although no epidemiological link between the United Kingdom and Hawaii ST649 strains has been established, ST649 could be more widely disseminated than previously thought. Consistent with reports from the United Kingdom and Argentina [15, 16], the strain from this study contained A2059G mutation in all 4 alleles, supporting the role of A2059G in high-level azithromycin resistance in *N. gonorrhoeae*. However, the Argentinean strain is ST696 [15] and hence is not implicated here.

Emerging antimicrobial resistance in *N. gonorrhoeae* is a major public health challenge. Hawaii was selected as a sentinel site for national gonococcal surveillance because it is considered a "port of import" for antimicrobial-resistant strains entering the United States [17]. Hawaii was one of the first states identified with high-level resistance to penicillin [18] and fluoroquinolones [19]. Gonococcal isolates in the United States demonstrating resistance to penicillin, tetracycline, and ciprofloxacin and reduced susceptibility to cefixime were first identified in Hawaii in 2001 [20]. In Hawaii, during 2010, of 216 isolates of *N. gonorrhoeae* tested, 87.0% were susceptible to cefpodoxime (MIC ≤0.5 μg/mL) and 99.1% were susceptible to azithromycin (MIC ≤2.0 μg/mL). Ceftriaxone and cefixime were the only antibiotics for which isolates demonstrated 100% susceptibility (HDOH, unpublished data).

Third-generation cephalosporins are now considered the only class of antibiotics that are well-studied and effective against *N. gonorrhoeae* infections in the United States [21]. Dual therapy with a cephalosporin (ceftriaxone is preferred) and either azithromycin (preferred) or doxycycline is recommended by CDC for treatment of gonorrhea in adults [22]. Although azithromycin monotherapy is not recommended due to concerns about the ease with which *N. gonorrhoeae* can develop macrolide resistance [22], 2 g of oral azithromycin is sometimes used for gonorrhea treatment in patients with severe cephalosporin allergy. The recent CDC "Dear Colleague" letter advises that if azithromycin monotherapy is used to treat gonorrhea, a test of cure (preferably culture-based) should be performed in 1 week [3].

A recent GISP report documented an increasing trend of gonococcal isolates in the United States with elevated MICs of cefixime (≥0.25 μg/mL) from 0.2% in 2000 to 1.4% in 2010 and of ceftriaxone (≥0.125 μg/mL) from 0.1% in 2000 to 0.3% in 2010 [23]. The current isolate, although cefixime susceptible, has an MIC 1 dilution from the CLSI breakpoint for susceptibility. In 2009, the first gonococcal isolate with ceftriaxone resistance (MIC = 2 μg/mL) was identified in Japan [24]. The emergence of both cephalosporin and macrolide resistance would severely limit our treatment options for gonorrhea and could pose a potential crisis if a single strain emerged with resistance to both antimicrobial classes.

Gonococcal antibiotic susceptibility testing relies on culture specimens. With most gonorrhea testing now performed by NAATs, the initial emergence and spread of antimicrobial resistance may go unrecognized. As noted by CDC, "to detect antibiotic resistance beyond GISP and guide treatment of gonorrhea, it is essential that gonorrhea culture and susceptibility testing capacity be expanded at the local level, where it is waning, due, in part, to the widespread use of NAATs" [3]. Hawaii has been able to maintain culture capacity through the HDOH Gonorrhea Culture Screening Program. Established in 1972, this program provides free gonococcal culture–based screening services to selected clinics, including community health centers and the university health center. An important

component of this program is antibiotic susceptibility testing, which has unfortunately declined with fewer culture submissions coinciding with increased use of NAATs. Strategies to enhance gonococcal isolate and susceptibility surveillance nationally could be modeled after Hawaii's efforts and include health departments partnering with community health centers and college-based clinics. Health departments could also offer culture-based screening where clinical laboratories do not, or in cases where health plans do not cover testing by NAAT and culture on the same patient.

HDOH recommends that *N. gonorrhoeae* cultures be obtained before treatment from persons either treated presumptively or found to be positive for gonorrhea by NAAT. Patients with suspected treatment failures should have specimens obtained for culture and antimicrobial susceptibility testing [22] and should be promptly reported to the local or state health department and CDC [3].

This report serves as a reminder of the looming threat of multidrug-resistant *N. gonorrhoeae*. Continued gonorrhea prevention efforts, local development of surveillance and response plans, and maintenance of gonococcal culture capacity are warranted. Clinicians should maintain vigilance for gonorrhea treatment failures and promptly report treatment failures to the local or state health department and CDC. Drug developers are urged to prioritize the identification of new antibiotics for gonorrhea.

Notes

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References

1. Centers for Disease Control and Prevention. Gonococcal isolate surveillance project protocol. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, **2007**. Available at: www.cdc.gov/std/gisp/protocol2006_web_version_rev12_2007.pdf. Accessed 6 December 2011.
2. Hawaii State Department of Health. Medical advisory: first azithromycin-resistant gonorrhea isolate. **2011**. Available at: http://hawaii.gov/health/healthy-lifestyles/std-aids/azi.pdf. Accessed 6 December 2011.
3. Centers for Disease Control and Prevention. Dear colleague letter: azithromycin resistance in Hawaii. **2011**. Available at: http://www.cdc.gov/std/gonorrhea/DCL-Azithro-GC-May-24-2011.pdf. Accessed 6 December 2011.
4. Martin IM, Ison CA, Aanensen DM, Fenton KA, Spratt BG. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J Infect Dis **2004**; 189:1497–505.
5. Ng LK, Martin I, Liu G, Bryden L. Mutation in 23S rRNA associated with macrolide resistance in *Neisseria gonorrhoeae*. Antimicrob Agents Chemother **2002**; 46:3020–5.
6. Luna VA, Cousin S Jr, Whittington WL, Roberts MC. Identification of the conjugative mef gene in clinical *Acinetobacter junii* and *Neisseria gonorrhoeae* isolates. Antimicrob Agents Chemother **2000**; 44:2503–6.
7. Roberts MC, Chung WO, Roe D, et al. Erythromycin-resistant *Neisseria gonorrhoeae* and oral commensal *Neisseria* spp. carry known rRNA methylase genes. Antimicrob Agents Chemother **1999**; 43:1367–72.
8. Cousin SL Jr, Whittington WL, Roberts MC. Acquired macrolide resistance genes and the 1 bp deletion in the *mtrR* promoter in *Neisseria gonorrhoeae*. J Antimicrob Chemother **2003**; 51:131–3.
9. Centers for Disease Control and Prevention. *Neisseria gonorrhoeae* with reduced susceptibility to azithromycin—San Diego County, California, 2009. MMWR Morb Mortal Wkly Rep **2011**; 60:579–81.
10. Galarza PG, Alcalá B, Salcedo C, et al. Emergence of high level azithromycin-resistant *Neisseria gonorrhoeae* strain isolated in Argentina. Sex Transm Dis **2009**; 36:787–8.
11. Palmer HM, Young H, Winter A, Dave J. Emergence and spread of azithromycin-resistant *Neisseria gonorrhoeae* in Scotland. J Antimicrob Chemother **2008**; 62:490–4.
12. Chisholm SA, Neal TJ, Alawattegama AB, Birley HD, Howe RA, Ison CA. Emergence of high-level azithromycin resistance in *Neisseria gonorrhoeae* in England and Wales. J Antimicrob Chemother **2009**; 64:353–8.
13. Cole MJ, Chisholm SA, Hoffmann S, et al. European surveillance of antimicrobial resistance in *Neisseria gonorrhoeae*. Sex Transm Infect **2010**; 86:427–32.
14. Starnino S, Stefanelli P. Azithromycin-resistant *Neisseria gonorrhoeae* strains recently isolated in Italy. J Antimicrob Chemother **2009**; 63: 1200–4.
15. Galarza PG, Abad R, Canigia LF, et al. New mutation in 23S rRNA gene associated with high level of azithromycin resistance in *Neisseria gonorrhoeae*. Antimicrob Agents Chemother **2010**; 54:1652–3.
16. Chisholm SA, Dave J, Ison CA. High-level azithromycin resistance occurs in *Neisseria gonorrhoeae* as a result of a single point mutation in the 23S rRNA genes. Antimicrob Agents Chemother **2010**; 54: 3812–6.
17. Gorwitz RJ, Nakashima AK, Moran JS, Knapp JS. Sentinel surveillance for antimicrobial resistance in *Neisseria gonorrhoeae*—United States, 1988–1991. The Gonococcal Isolate Surveillance Project Study Group. MMWR CDC Surveill Summ **1993**; 42:29–39.
18. Sarafian SK, Rice RJ, Ohye RG, Higa H, Knapp JS. Diversity of isolates of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) in Honolulu, Hawaii: 1982–1991. Sex Transm Dis **1994**; 21:332–7.
19. Knapp JS, Ohye R, Neal SW, Parekh MC, Higa H, Rice RJ. Emerging in vitro resistance to quinolones in penicillinase-producing *Neisseria gonorrhoeae* strains in Hawaii. Antimicrob Agents Chemother **1994**; 38:2200–3.
20. Wang SA, Lee MVC, O'Connor N, et al. Multidrug-resistant *Neisseria gonorrhoeae* with decreased susceptibility to cefixime—Hawaii, 2001. Clin Infect Dis **2003**; 37:849–52.
21. Centers for Disease Control and Prevention. Consultation meeting on cephalosporin-resistant gonorrhea outbreak response plan. **2009**. Available at: http://www.cdc.gov/std/Gonorrhea/arg/CephOutbreakResponsePlan.pdf. Accessed 6 December 2011.
22. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep **2010**; 59:49–55.
23. Centers for Disease Control and Prevention. Cephalosporin susceptibility among *Neisseria gonorrhoeae* isolates—United States, 2000–2010. MMWR Morb Mortal Wkly Rep **2011**; 60:873–7.
24. Ohnishi M, Saika T, Hoshina S, et al. Ceftriaxone-resistant *Neisseria gonorrhoeae*, Japan. Emerg Infect Dis **2011**; 17:148–9.