



Published in final edited form as:

*Sex Transm Dis.* 2015 May ; 42(5): 279–280. doi:10.1097/OLQ.0000000000000265.

## Failure of Azithromycin 2.0 g in the Treatment of Gonococcal Urethritis Caused by High-Level Resistance in California

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### Abstract

We report a treatment failure to azithromycin 2.0 g caused by a urethral *Neisseria gonorrhoeae* isolate with high-level azithromycin resistance in California. This report describes the epidemiological case investigation and phenotypic and genetic characterization of the treatment failure isolate.

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In May 2014 (day 0), a 23-year-old heterosexual white man (index patient) presented to a clinic in California with a 2-day history of dysuria and a small amount of urethral discharge. He was treated with azithromycin (AZM) 1.0 g orally in a single dose for presumed nongonococcal urethritis, and a urine specimen was obtained for nucleic acid amplification testing (NAAT) for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. On day 2, the NAAT returned positive for *N. gonorrhoeae*, and the index patient was treated the same day with AZM 2.0 g orally in a single dose. Azithromycin monotherapy was given rather than the Centers for Disease Control and Prevention (CDC)–recommended dual-therapy regimen (intramuscular [IM] ceftriaxone 250 mg in a single dose plus either AZM 1.0 g orally or doxycycline 100 mg orally twice a day for 7 days) because the index patient reported developing a rash after taking penicillin as a child. The index patient’s symptoms persisted, and he was seen in the clinic again on day 8, at which time a urethral specimen was obtained for culture given the clinician’s concerns about possible AZM resistance. The culture was presumptively identified as *N. gonorrhoeae*, and the isolate was sent to the San Francisco Department of Public Health Laboratory for species confirmation, antimicrobial

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Conflicts of interest

All authors have no conflicts to declare.

susceptibility testing, and molecular characterization. On day 12, the index patient was treated with ceftriaxone 250 mg IM, which he tolerated well with no allergic reaction. On day 14, the index patient reported improvement in his symptoms.

The isolate's presumptive identification was confirmed by the San Francisco Department of Public Health Laboratory based on NAAT (Aptima Combo 2; Hologic Inc, Bedford, MA) and a species-specific biochemical test (API NH; BioMérieux Clinical Diagnostics, Marcy l'Etoile, France), and the strain was designated CA-1461. Antimicrobial susceptibility testing using Etest (BioMérieux Clinical Diagnostics) revealed that CA-1461 possessed an AZM minimum inhibitory concentration (MIC) greater than 256 µg/mL.

CA-1461 was further characterized by the *Neisseria* Reference Laboratory at the University of Washington, Seattle. The *Neisseria* Reference Laboratory confirmed the identification of CA-1461 as *N. gonorrhoeae* based on culture morphology, Gram stain, superoxol test, colistin resistance test, oxidase test, and 3 species confirmatory tests—acid detection test, PhadeBact GC monoclonal test (MKL Diagnostics AB, Sollentuna, Sweden), and API NH. Antimicrobial susceptibility testing was also conducted using the Clinical and Laboratory Standards Institute–recommended agar dilution method<sup>1</sup> and 7 CDC quality control *N. gonorrhoeae* strains, as described previously.<sup>2</sup> CA-1461 displayed high-level resistance to AZM (>2048 µg/mL), intermediate susceptibility to penicillin (0.25 µg/mL) and tetracycline (1.0 µg/mL), and susceptibility to spectinomycin (16 µg/mL), gentamicin (4 µg/mL), cefixime (0.015 µg/mL), ceftriaxone (0.008 µg/mL), and ciprofloxacin (0.015 µg/mL). Minimum inhibitory concentrations for all antimicrobials were interpreted using the 2014 Clinical and Laboratory Standards Institute criteria,<sup>3</sup> with the exception of AZM<sup>4</sup> and gentamicin<sup>5</sup> where the referenced criteria were used.

Based on the patient's clinical history and CA-1461's high AZM MIC, a field investigation was initiated by the local health department. The index patient reported that his symptoms started shortly before diagnosis in May 2014 and denied any sexual contact for 1 month after he first developed symptoms. His female partner was treated as a contact, but testing for *N. gonorrhoeae* was not done. Her previous male partner was asymptomatic and had no male sexual partners.

The index patient, his female partner, and her previous male partner were seen in July for follow-up testing. The index patient was negative for *N. gonorrhoeae* by urine NAAT and urethral culture. His female partner was negative for *N. gonorrhoeae* of the pharynx, vagina, and rectum by NAAT. Her previous male partner was treated as a contact, and the specimen collected from him before treatment was negative for *N. gonorrhoeae* by urine NAAT and urethral culture. All 3 individuals were negative for HIV and syphilis.

CA-1461 was characterized by *N. gonorrhoeae* multiantigen sequence typing<sup>6</sup> and screened for AZM resistance–associated mutations by sequencing the peptidyltransferase region of domain V of the 23S rRNA *rrl* gene,<sup>7</sup> the *mtrR* promoter region, and the *mtrR* coding region,<sup>2</sup> as described previously. In addition, polymerase chain reaction assays were performed to detect the presence of the efflux pump *mef(A)* gene and methylase-encoding *erm(A)*, *erm(B)*, and *erm(C)*, as described previously.<sup>8</sup>

*N. gonorrhoeae* multiantigen sequence typing revealed that CA-1461 was a novel sequence type (ST), and ST10844 (*tbpB* 29, *por* 6354) was assigned by the *N. gonorrhoeae* multiantigen sequence typing database (<http://www.ng-mast.net/>). The strain contained an A2143G (*N. gonorrhoeae* numbering) mutation in all 4 alleles of the 23S rRNA *rrl* gene and a G45D mutation in the *mtrR* coding region. No mutations were detected in the *mtrR* promoter region, and the isolate was negative for *mef*(A), *erm*(A), *erm*(B), and *erm*(C).

This is the first report of a treatment failure caused by a gonococcal strain with high-level resistance (>2048 µg/mL) to AZM in the United States. No pretreatment isolate was available in this case, but the index patient denied any sexual contact after his initial clinic visit. A previous case of unsuccessful treatment with AZM was reported in Oregon in 2011, but the strain responsible for that case only displayed moderate AZM resistance (MIC 8–16 µg/mL).<sup>2</sup> Isolates with moderate AZM resistance have also been reported in California,<sup>9</sup> but CA-1461 is the first report of a strain with high-level AZM resistance in California. Fortunately, CA-1461 was fully susceptible to both cefixime (MIC 0.015 µg/mL) and ceftriaxone (MIC 0.008 µg/mL) and could therefore have been successfully treated with the CDC-recommended dual-therapy regimen, which includes ceftriaxone 250 mg IM.

Similar to other gonococcal strains with high-level resistance that have been reported in Hawaii,<sup>4</sup> Canada,<sup>10</sup> the United Kingdom,<sup>11–13</sup> and Argentina,<sup>14,15</sup> CA-1461 contained the A2143G mutation in all 4 alleles of the 23S rRNA *rrl* gene, which further supports its association with high-level AZM resistance. CA-1461 also contained the G45D mutation that has previously been associated with moderate AZM resistance.<sup>16</sup>

CA-1461 was assigned ST10844, an ST composed of *por* allele 6354 and *tbpB* allele 29. Although this ST was novel, sequence analysis revealed that it was closely related to the ST649 strains (*tbpB* 29, *por* allele 442, 1-bp difference) with high-level AZM resistance that have been reported in the United Kingdom and Hawaii. Interestingly, these ST649 strains were also detected in young, heterosexual populations and were not associated with the core groups such as men who have sex with men that have been associated with emerging resistance in *N. gonorrhoeae*.<sup>17</sup> A recent longitudinal study of AZM resistance in the United States found that men who have sex with men had significantly higher AZM MIC geometric means compared with heterosexual men, but found no overall temporal trend in AZM MIC geometric means from 2005 to 2013.<sup>18</sup> The ST696 strain detected in Argentina also possessed *tbpB* allele 29, but had a *por* allele that was not closely related to *por* 6354. In this case, the field investigation was unable to determine the origin of CA-1461 and no recent travel outside California was reported by any of the contacts interviewed.

This case highlights the importance of clinical vigilance for treatment failures and provides an excellent example of collaboration between clinicians and public health. In this case, the treating physician recognized the increased risk of treatment failure with the use of AZM monotherapy, performed a test-of-cure culture given persistent symptoms after treatment, and contacted local public health authorities when a treatment failure was suspected per California Department of Public Health guidelines.<sup>19</sup> Although most *N. gonorrhoeae* infections in the United States are diagnosed by NAAT, this case also demonstrates how culture remains a critical tool for the surveillance of antimicrobial susceptibilities. *N.*

*gonorrhoeae* has proven its ability to evolve or acquire the adaptations necessary to escape antimicrobial pressure. Continued surveillance will be necessary to further investigate the spread of strains with high-level AZM resistance in the United States.

## Acknowledgments

The authors thank Geri Beaman (San Luis Obispo Department of Public Health), Winston Tilghman, and Heidi Aiem (County of San Diego Health & Human Services Agency) for their assistance with this case.

source of funding: The San Francisco Public Health Laboratory and the *Neisseria* Reference Laboratory are supported, in part, by the Centers for Disease Control and Prevention (PS001411-03).

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