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Sustained Transmission of *Neisseria gonorrhoeae* with High-Level Resistance to Azithromycin, in Indianapolis, Indiana, 2017–2018

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Abstract

Background: Since 2014, *Neisseria gonorrhoeae* azithromycin (AZM) susceptibility has declined in the United States, but high-level AZM resistance (HL-AZMR) has been infrequent and sporadic. We describe a cluster of 14 *N. gonorrhoeae* isolates with HL-AZMR identified in Indianapolis over 13 months.

Methods: *N. gonorrhoeae* culture specimens (genital and extragenital) were collected from attendees of the Bell Flower Clinic. Isolates underwent antimicrobial susceptibility testing (AST) using Etest. AZM minimum inhibitory concentrations ≥256 μg/mL were classified as HL-AZMR. Local disease intervention specialists interviewed patients whose isolates demonstrated HL-AZMR and conducted partner services. Relatedness of isolates was investigated by genomic analyses.

Results: During 2017–2018, AST was performed in 1016 *N. gonorrhoeae* isolates collected at the Bell Flower Clinic. Fourteen isolates (1.4%) from 12 men collected over 13 months demonstrated HL-AZMR; all were cephalosporin susceptible. Of the 12 men, 9 were white and reported male sex partners. Nine of the men were able to be retested; all were cured with 250-mg ceftriaxone plus 1-g AZM. Two men named each other as partners; no other partners in common were reported. Genomic analysis demonstrated close relatedness of the HL-AZMR isolates and a novel combination of a mosaic-*mtr*R promoter along with 23S ribosomal RNA mutations that appear to have emerged from circulating strains.

Conclusions: The close genetic relatedness with limited epidemiologic linkages between patients highlights the challenges of gonorrhea partner investigations and suggests undetected

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local transmission. Local AST, rapid public health action, and epidemiologic investigations combined with genomic analysis provides a multipronged approach to understanding an outbreak of sexually transmitted disease.

Keywords

Neisseria gonorrhoeae ; gonorrhea; antimicrobial resistance; azithromycin; whole-genome sequencing

Rates of *Neisseria gonorrhoeae* infections in the United States increased 63% from 2014–2018, when 583 405 cases were reported [1]. Throughout the 20th century, *N. gonorrhoeae* developed antimicrobial resistance to multiple antibiotic classes [2]. The Centers for Disease Control and Prevention (CDC) and the World Health Organization have labeled gonococcal antimicrobial resistance an urgent public health threat [3, 4]. Declining cephalosporin susceptibility has been observed in multiple countries [5], and a small number of persons globally have been unsuccessfully treated owing to documented ceftriaxone resistance [6, 7].

Compounding concern about emerging cephalosporin resistance, azithromycin (AZM) susceptibility has declined in multiple countries [8–11]. In the United States, the percentage of isolates in the Gonococcal Isolate Surveillance Project (GISP) with elevated AZM minimum inhibitory concentrations (MIC) (≥2 μg/mL) increased from 0.6% to 4.6% between 2013 and 2018 [12]. During 2015–2020, ceftriaxone plus AZM was the only first-line treatment recommended by the CDC for uncomplicated gonococcal infections [13]. With the decrease in AZM susceptibility, and an emphasis on antimicrobial stewardship, the CDC no longer recommends AZM in treatment of uncomplicated gonorrhea infection [14]. Yet AZM remains a component of back-up treatment in the United States and is recommended by the World Health Organization as part of dual therapy for gonorrhea [15].

Isolates with high-level AZM resistance (HL-AZMR) (MIC, ≥256 μg/mL) have been sporadically identified in the United States, though they appear to be increasing in frequency. Isolates were detected in Hawaii in 2011 [16], in Baltimore in 2016 [17], and in North Carolina in 2018 [18]. CDC surveillance projects have identified additional isolates in San Francisco, Seattle, New York City, and Denver between 2017 and 2020 (unpublished data). A cluster of 7 patients whose isolates demonstrated both HL-AZMR and reduced ceftriaxone susceptibility (MIC, ≥0.125 μg/mL) was identified during April and May 2016 in Hawaii [19]. After patient treatment and rapid contact tracing, the observed strain was no longer detected. When *N. gonorrhoeae* isolates or clusters with HL-AZMR have been detected they seem to appear briefly and then rapidly disappear.

Although detection of gonococcal infections with HL-AZMR has been infrequent in the United States, the Indiana Department of Health identified 14 HL-AZMR isolates over a 13-month period beginning in September 2017. We describe the epidemiology, microbiology, and genomics of the infections and summarize the resulting case investigations.

METHODS

Isolate Collection and Susceptibility Testing

The Indiana Department of Health and the Marion County Public Health Department participate in the CDC-supported Strengthening the US Response to Resistant Gonorrhea (SURRG) program [20], a multisite project designed to enhance local preparedness for antimicrobial-resistant *N. gonorrhoeae* through expanded culture collection, local antimicrobial susceptibility testing (AST), and enhanced epidemiologic and field investigation capacity. Beginning May 2017, specimens for *N. gonorrhoeae* culture were collected within the county's sexually transmitted disease (STD) clinic and in other health-care settings (a human immunodeficiency virus testing/services center, 2 emergency departments, and an infectious disease clinic). Genital, rectal, and pharyngeal specimens for *N. gonorrhoeae* culture were collected from patients of all genders at all anatomic sites of exposure who (1) presented with mucopurulent genital discharge from suspected gonorrhea, (2) had gonorrhea diagnosed by means of nucleic acid amplification testing (NAAT), or (3) were a recent sex partner of a person with diagnosed gonorrhea.

At the local public health laboratory, AST was performed on all gonococcal isolates using Etest for ceftriaxone, cefixime, and AZM [21]. Isolates were shipped to the Tennessee Department of Health Laboratory Services, a regional laboratory in the CDC-funded Antibiotic Resistance Laboratory Network [22], for AST on an expanded antimicrobial panel by agar dilution. Isolates found to have HL-AZMR by Etest were retested at CDC with both Etest and agar dilution.

Clinical and Laboratory Standards Institute (CLSI) defines isolates with AZM MICs of >1 μg/mL as nonsusceptible [23]. For this analysis, nonsusceptible isolates were further classified as exhibiting reduced susceptibility to AZM (AZM-RS) (2–128 μg/mL) or HL-AZMR (≥256 μg/mL). We classified resistance to penicillin (≥2 μg/mL), ciprofloxacin (≥1 μg/mL), and tetracycline (≥2 μg/mL) using CLSI criteria [23], and gentamicin > 8 μg/mL as nonsusceptible [24]. CLSI defines ceftriaxone and cefixime nonsusceptibility as >0.25 μg/mL [23]; SURRG protocols use MIC thresholds of reduced susceptibility to ceftriaxone and cefixime of ≥0.125 and ≥0.25 μg/mL, respectively, to more proactively detect emerging resistance and react to potential public health threats.

Whole-Genome Sequencing

All isolates with HL-AZMR identified from Indiana underwent whole-genome sequencing (WGS) and genomic analyses. In addition, genomic data from a convenience sample of other isolates from Indiana (chosen for sequencing to support other projects) were included [25]. Isolates within the convenience sample were collected from March 2016 to June 2018 and fit within specified categories (ie, first 5 urethral isolates from men collected each month, isolates with AZM MICs ≥2 μg/mL, ceftriaxone MICs ≥0.125 μg/mL, or cefixime MICs ≥0.25 μg/mL, and extragenital isolates). WGS of the specimens was completed by the Antibiotic Resistance Laboratory Network; raw data were sent to the CDC for assembly and analysis of multilocus sequence typing (MLST), antimicrobial resistance determinants, and

phylogenetic analysis, according to a previously established GISP WGS analysis protocol [25].

Case Investigations

Patients with gonorrhea diagnosed at the STD clinic are routinely interviewed by disease intervention specialists (DISs), including elicitation of recent sexual partners, risk reduction counseling, and referral to services, including preexposure prophylaxis and substance use. As part of SURRG, identification of an isolate with AZM-RS, HL-AZMR, or reduced ceftriaxone or cefixime susceptibility from participating clinics triggered an enhanced case investigation. During an enhanced investigation, DISs collected detailed information, including recent use of geospatial apps to find partners, number of recent partners, sex with anonymous partners, and recent travel, and encouraged the patient to return for a test of cure (NAAT and culture specimen collection at infected anatomic sites) 8–10 days after treatment. If patients provided sufficient contact information, DISs attempted to contact recent sexual partners. Partners were referred to a participating SURRG clinic for testing and presumptive treatment. As part of the rapid detection and containment approach of SURRG, these partners—regardless of whether they were infected—were interviewed to elicit their recent sexual partners. DISs then attempted to contact, interview, and arrange testing for these recent sexual partners. Partner elicitation and interviewing would continue until 2 generations of partners were found to be negative for a gonococcal infection. For this analysis, we focused on the partner investigations initiated by patients with HL-AZMR.

Statistical Analysis

Epidemiologic, clinical, and laboratory data were extracted from medical records and surveillance systems. Because there were no transgender patients, we classified the cisgender patients as either male or female based on the patients' gender identification. Information on the number and sex of sex partners within the past 60 days was gathered. We classified men who reported sex with only male (cisgender or transgender) partners as men who have sex with men (MSM), men who reported sex with only female (cisgender or transgender) partners as men who have sex with women (MSW), and men who reported sex with both male and female partners as men who have sex with men and women (MSMW). Anonymous sexual partners were those with no identifiable information. Unnamed partners were defined as such if the patient refused to provide contact or identifying information.

We used χ^2 testing to compare patient characteristics (demographics, number of partners in past 60 days, use of geospatial apps, and anonymous partners) between those whose *N. gonorrhoeae* isolates were classified as AZM-RS and those with HL-AZMR isolates. Statistical tests were performed using SAS 9.4 software.

RESULTS

Neisseria gonorrhoeae Isolates

From May 2017–December 2018, 2042 *N. gonorrhoeae* culture samples were collected from participating SURRG clinics in Indiana. *N. gonorrhoeae* was isolated from 1024 (50.1%) of

the samples; 1016 isolates underwent AST. Most isolates (88.5%) with AST were urethral; 4.7% were pharyngeal, 3.6% were endocervical, and 3.1% were rectal.

Of the 1016 isolates that underwent local AST by Etest, 49 (4.8%) exhibited AZM-RS and an additional 14 (1.4%) demonstrated HL-AZMR; all demonstrated susceptibility to cefixime and ceftriaxone. Twenty-seven patients had isolates from multiple anatomic sites for the same gonococcal diagnosis; in such cases AST results were all within the same AZM resistance category.

Among the 14 isolates with HL-AZMR, 11 were urethral, 2 were pharyngeal, and 1 was a rectal specimen. The 14 isolates were collected from 12 patients (2 patients had concurrent urethral and pharyngeal isolates) from September 2017 to September 2018 (Figure 1). Agar dilution confirmed the HL-AZMR results with MICs ≥16 μg/mL, the end point for this method. Agar dilution also confirmed that all isolates with HL-AZMR demonstrated susceptibility to cefixime, ceftriaxone, penicillin, ciprofloxacin, and gentamicin; all were tetracycline resistant (MICs, 2–4 μg/mL).

Patient Characteristics

Table 1 details the 987 patients from whom the 1016 isolates were collected. All were cisgender, and 94.8% were men. Among men, 75.6% were MSW, 17.6% were MSM, and 4.9% were MSMW; data were unavailable for 20 men (1.9%). A total of 695 (70.4%) patients were non-Hispanic black, and 230 (23.3%) were non-Hispanic white.

All 12 patients with HL-AZMR were men who attended the STD clinic; 7 were MSM, 2 were MSMW, and 3 were MSW. Men with HL-AZMR were significantly more likely to report sex with male partners (75.0%) than men with AZM-RS (32.5%) ($P = .02$) and were more often white (75.0% and 31.9%, respectively). Although higher percentages of patients with HL-AZMR reported use of geospatial apps (41.7%), multiple partners (41.7%), and anonymous partners (50%) than patients with AZM-RS (27.7%, 19.1%, and 42.6%, respectively), the differences were not significant. Seven patients with HL-AZMR had prior gonococcal infections; 1 reported AZM use within the previous 60 days.

No patients with HL-AZMR reported having sexual partners who had recently traveled. One patient reported recent travel outside Indiana: this patient traveled from California and had been in Indiana for 2–3 weeks before presenting to the STD clinic. While in Indiana, he had 2 anonymous sexual encounters, with the most recent occurring 2 days before he presented with urethral discharge. This patient spoke with his regular partner in California, who was asymptomatic. Based on sexual exposure time periods, onset of symptoms, and length of stay, the Indiana STD program concluded that this patient most likely obtained his infection while in Indiana.

Nine of the 12 men returned to the clinic for test of cure, and all 9 were negative for *N. gonorrhoeae* at follow-up. One was asymptomatic and the remaining 8 had urethral discharge at their initial appointment which resolved following treatment with ceftriaxone plus AZM. Of the 3 men who did not return (all had urethral isolates, and 2 also had

pharyngeal isolates with HL-AZMR), 1 had returned to California and 2 refused further services.

Local Case Investigations

The 12 men with HL-AZMR isolates reported a total of 31 partners within the previous 60 days and provided sufficient identifying information for 13 of them (41.9%), who included both men and women (Figure 2). Two men with HL-AZMR named each other and a common male partner (Figure 2, labeled 1 and 2), so 11 unique partners were pursued. Nine of the 11 partners were able to be contacted, tested (only 1 had a specimen collected for culture), and treated presumptively; 3 of the 9 tested positive for gonorrhea by NAAT. Three male partners who tested negative for gonorrhea reported 9 additional recent partners. Four of these 9 were able to be contacted and tested; 2 tested positive for gonorrhea by NAAT. Both were found during a screening event held by DISs at a local venue named by multiple patients as a place to meet partners.

Genomic Analysis

WGS was performed on 119 isolates collected in Indiana during 2016–2018 through the GISP and SURRG programs. Figure 3 displays the phylogenetic analysis of the sequenced isolates. Clade A (A1 and A2) contained almost all isolates with AZM-RS and all isolates with HL-AZMR; 29.7% of isolates in clade A (19 of 64) had mutations associated with AZM-RS [26], including a mosaic *mtrR*-coding region and either a C-substitution or A-deletion in the *mtrR* promoter. MLST analysis revealed that 39.1% of isolates in clade A (25 of 64) belonged to the MLST ST9363. The HL-AZMR isolates had similar mosaic-*mtr* locus mutations, as well as mutations in all 4 copies of the 23S ribosomal RNA (rRNA) A2059G gene.

Overall, the isolates in clade A differed by 46–74 single nucleotide polymorphisms (SNPs). The HL-AZMR isolates (clade A1) differed by 4–6 SNPs. Isolates from 2 patients with HL-AZMR who named each other as sexual partners differed by 5 SNPs (Figure 3; GCWGS-2471 and GCWGS-2472, highlighted in green). Another pair of isolates collected from sexual partners was identified: the isolates demonstrated AZM susceptibility and differed by 46 SNPs (Figure 3; isolates GCWGS-2580 and GCWGS-2600, highlighted in red).

DISCUSSION

We identified a cluster of 14 *N. gonorrhoeae* isolates with HL-AZMR from 12 patients over a 13-month period in Indianapolis, Indiana. Detection of a HL-AZMR cluster over such an extended period has not been previously reported in the United States. HL-AZMR was seen primarily in white men reporting male partners. Half of these patients reported anonymous partners, and 41.5% reported use of geospatial apps to meet partners. This was different than other groups of AZM susceptibility.

Although only 2 patients reported partners in common, genomic analyses indicated that this cluster of isolates had a high degree of genetic relatedness, suggesting recent and sustained transmission within an Indianapolis sexual network that included MSM, MSMW, MSW,

and women. Available data suggest that the HL-AZMR strain was not imported, but rather emerged from an MLST commonly seen in GISP data [27] (ST9363) (clade A). This MLST was prevalent in the sequenced isolates from other GISP studies [25, 28] and was commonly associated with low-level AZM-RS via mosaic-*mtrR* mutations hypothesized to increase the survivability of the bacteria [27]. In Indiana, ST9363 mutations were seen in isolates as far back as 2016.

Thomas et al [25] reported that A2059G mutations in all 4 copies of the 23S rRNA gene were commonly associated with other HL-AZMR isolates, although with differing MLST. The A2059G mutations tend to be sporadic and may "carry a fitness cost" [27]. The observed HL-AZMR seems to be due to the more recent acquisition of these A2059G mutations. This novel combination of mutations affecting AZM susceptibility in these isolates was first reported by Pham et al [29]. This genotypic combination may further improve the fitness of this HL-AZM strain through the stability of ST9363 mutation [30].

Previous genomic studies have found that sequences of *N. gonorrhoeae* isolates from named sexual partners vary by 0–10 SNPs [31, 32]. All isolates with HL-AZMR in our study differed by 4–6 SNPs, suggesting recent transmission within a closely linked sexual network. However, only 2 people identified each other as recent sexual partners. The small number of partners investigated prevent us from truly understanding the sexual networks. Missing partners may provide connections between disparate clusters.

Isolates from another pair of named partners (Figure 3, highlighted in red) that did not possess HL-AZMR revealed a larger SNP difference of 46. Although they had named each other as partners, it is possible (given the SNP difference) that transmission either did not occur between them, that transmission was not recent, or that this was a mixed infection. Epidemiologic data provide a network of partners but do not tell the whole story. This pair highlights the complexities of sexual networks and the potential strength of analyzing networks through both WGS and epidemiologic methods.

An outbreak of 16 HL-AZMR gonococcal infections was previously detected in Leeds, North England, between November 2014 and October 2015 [33, 34]. In contrast to the Indiana cluster, the Leeds outbreak occurred among heterosexuals (only later spreading to MSM). However, the Leeds and Indiana outbreaks otherwise have similar hallmarks: sustained transmission, HL-AZMR isolates demonstrating cephalosporin susceptibility, clonality by genomic analysis, and A2059G mutations in all copies of the 23S rRNA gene. In addition, partner notification was noted by the authors to have had limited success, because only 33 of 248 partners were verified to have been contacted and/or tested [34].

During the investigation of the Indianapolis cluster, DISs experienced challenges eliciting sex partner contact information. Only 2 of the 12 patients with HL-AZMR infections named each other as recent sexual partners, and DISs were able to contact only 9 of the 31 partners. The relative paucity of contacted partners highlights inherent challenges in containing the spread of resistant gonococcal strains through partner investigations, even with use of enhanced techniques, such as investigating partners of partners. Patients may be unwilling to divulge the contact information of recent partners. Yet even if patients are

willing to provide information, sex with anonymous partners and use of geospatial apps to find partners (as was common among those with HL-AZMR) can limit their ability to provide sufficient identifying information to DISs. The limited number of patients who named each other as partners or who named persons in common might also indicate that transmission of this strain was somewhat widespread within the community. With almost half of the HL-AZMR patients mentioning geospatial apps, leveraging these systems to find partners, along with increased screening events at commonly reported venues, and incorporating genomic analyses and molecular epidemiology in identifying clusters and sexual networks holds promise for increasing the effectiveness of investigations.

The current study has limitations. These data are from a single location that implemented enhanced *N. gonorrhoeae* antimicrobial resistance detection and response; results may not be generalizable. Culture growth was suboptimal in specimens from extragenital sites, limiting identification of resistance in such isolates. Collection of specimens for culture from sexual partners proved challenging, limiting detection of resistance, and limiting elucidation of a more complete sexual network. Relatively limited culture- and sequencing-based surveillance before 2016 and the use of a convenience, nonsystematically collected sample in the phylogenetic analysis may have not detected earlier acquisition of these mutations.

After sustained detection of HL-AZMR for more than a year, transmission seems to have slowed or halted. Only a single isolate with HL-AZMR was identified in 2019 and none in 2020. Although relatively few partners were contacted, the rapid detection and response approach might have been enough to slow transmission. Alternatively, slowed or halted transmission might have been due to infections occurring in persons who did not infect others, or the fitness cost of the 23S mutation may still be too high for continued propagation. Studies such as this highlight how local AST and rapid public health responses provide timely warning of potential threats, and the incorporation of epidemiologic investigations with genomic analysis may provide a further understanding of STD outbreaks.

Responding to the threat of emerging gonococcal resistance will require a multipronged approach. Enhanced surveillance of *N. gonorrhoeae* susceptibility should continue, including maintaining access to culture and AST, ensuring adherence to screening and treatment recommendations, and improving detection of resistance through clinical vigilance. Slowing the spread of potentially resistant *N. gonorrhoeae* strains with innovative approaches to identifying patients within a sexual network and the application of genomic epidemiology to field investigations may allow time for development of additional prevention approaches.

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References

1. Centers for Disease Control and Prevention. Sexually transmitted disease surveillance 2018. Available at: https://www.cdc.gov/std/stats18/default.htm. Accessed 31 October 2019.
2. Unemo M, Seifert HS, Hook EW 3rd, Hawkes S, Ndowa F, Dillon JR. Gonorrhoea. Nat Rev Dis Primers 2019; 5:79. [PubMed: 31754194]
3. Centers for Disease Control and Prevention. Antibiotic/antimicrobial resistance (AR/AMR). Biggest threats and data: 2019 AR threats report Available at: https://www.cdc.gov/drugresistance/biggest-threats.html. Accessed 13 December 2019.
4. Wi T, Lahra MM, Ndowa F, et al. Antimicrobial resistance *in Neisseria gonorrhoeae:* global surveillance and a call for international collaborative action. PLos Med 2017; 14:e1002344. [PubMed: 28686231]
5. Public Health England. Surveillance of antimicrobial resistance in Neisseria gonorrhoeae in England and Wales: key findings from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP). Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/746261/GRASP_2017_report.pdf. Accessed: 11 April 2019.
6. Eyre D, Sanderson ND, Lord E, et al. Gonorrhoea treatment failure caused by a *Neisseria gonorrhoeae* strain with combined ceftriaxone and high-level azithromycin resistance, England, February 2018. Euro Surveill 2018; 23:1800323. [PubMed: 29991383]
7. Public Health England. Two cases of resistant gonorrhea diagnosed in the UK. Available at: https://www.gov.uk/government/news/two-cases-of-resistant-gonorrhoea-diagnosed-in-the-uk. Accessed 9 August 2019.
8. Williams DA, Fairley C, Howden B, et al. Trends and risk factors for antimicrobial-resistant *Neisseria gonorrhoeae*, Melbourne, Australia, 2007–2018. J Antimicrob Chemother 2019; 63:e01221–19.
9. Ibargoyen García U, Nieto Toboso MC, Montoya Azpeitia E, et al. Epidemiological surveillance study of gonococcal infection in Northern Spain. Enferm Infecc Microbiol Clin 2019; 38:59–64.
10. European Centre for Disease Prevention and Control. Gonococcal antimicrobial susceptibility surveillance in Europe—results summary 2017. Stockholm, Sweden: European Centre for Disease Prevention and Control, 2019.
11. Galaza P, Alcala B, Salcedo C, et al. Emergence of high-level azithromycin-resistant *Neisseria gonorrhoeae* strain isolated in Argentina. Sex Trans Dis 2009; 36:787–8.
12. Centers for Disease Control and Prevention. 2018 STD surveillance report—GISP data. 2019. Available at: https://www.cdc.gov/std/stats18/figures/29.htm. Accessed 24 November 2020.
13. Centers for Disease Control and Prevention. 2015 sexually transmitted diseases treatment guidelines: gonococcal infections. Available at: https://www.cdc.gov/std/tg2015/gonorrhea.htm. Accessed 12 June 2018.
14. St. Cyr S, Barbee L, Workowski KA, et al. Update to CDC's treatment guidelines for gonococcal infection, 2020. MMWR Morb Mortal Wkly Rep 2020; 69:1911–6. [PubMed: 33332296]
15. World Health Organization. WHO guidelines for the treatment of Neisseria gonorrhoeae. 2016. Available at: https://www.who.int/reproductivehealth/publications/rtis/gonorrhoea-treatment-guidelines/en/. Accessed 11 January 2021.
16. Katz A, Komeya A, Soge O, et al. *Neisseria gonorrhoeae* with high-level resistance to azithromycin: case report of the first isolate identified in the United States. Clin Inf Dis 2011; 54:841–3.
17. Melendez J, Hardick J, Barnes M, Page K, Gaydos C. Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates in Baltimore, Maryland, 2016: the importance of sentinel surveillance in the era of multi-drug-resistant gonorrhea. Antibiotics 2018; 7:77. [PubMed: 30126088]
18. Palavecino E, Kilic A, Schmerer M, et al. First case of high-level azithromycin-resistant *Neisseria gonorrhoeae* in North Carolina. Sex Transm Dis 2020; 47:326–8. [PubMed: 32073548]

19. Katz A, Komeya A, Kirkcaldy RD, et al. Cluster of *Neisseria gonorrhoeae* isolates with high-level azithromycin resistance and decreased ceftriaxone susceptibility, Hawaii, 2016. Clin Inf Dis 2017, 65:918–23.
20. Centers for Disease Control and Prevention. Combating the threat of antibiotic-resistant gonorrhea. 2018. Available at: https://www.cdc.gov/std/gonorrhea/arg/CARB_FACTSHEET-2018.pdf. Accessed 25 November 2020.
21. Liu H, Taylor TH, Jr, Pettus K, Trees D. Assessment of Etest as an alternative to agar dilution for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*. J Clin Microbiol 2014; 52:1435–40. [PubMed: 24554750]
22. Kersh E, Pham C, Papp J, et al. Laboratory capacity for *Neisseria gonorrhoeae* antimicrobial susceptibility testing and whole-genome sequencing through the CDC's antibiotic resistance laboratory network. J Clin Microbiol 2020; 58:e01461–19. [PubMed: 32024723]
23. Clinical and Laboratory Standards Institute. M100: performance standards for antimicrobial susceptibility testing, 2019. Available at: http://em100.edaptivedocs.net/Login.aspx?_ga=2.212281245.884637729.1609265574-1635654735.1609265574. Accessed 8 April 2020.
24. Brown LB, Krysiak R, Kamanga G, et al. *Neisseria gonorrhoeae* antimicrobial susceptibility in Lilongwe, Malawi, 2007. Sex Transm Dis 2010; 37:169–72. [PubMed: 19901860]
25. Thomas JC, Seby S, Abrams AJ, et al. Evidence of recent genomic evolution in gonococcal strains with decreased susceptibility to cephalosporins or azithromycin in the United States, 2014–2016. J Infect Dis 2019; 220:294–305. [PubMed: 30788502]
26. Ohneck E, Zalucki Y, Johnson P, et al. A novel mechanism of high-level, broad-spectrum antibiotic resistance caused by a single base pair change in *Neisseria gonorrhoeae*. MBio 2011; 2:e00187–11. [PubMed: 21933917]
27. Schmerer MW, Abrams AJ, Seby S, et al. Genomic characterization of *Neisseria gonorrhoeae* strains from 2016 U.S. sentinel surveillance displaying reduced susceptibility to azithromycin. Antimicrob Agents Chemother 2020; 64:e02420–19. [PubMed: 32071056]
28. Grad Y, Harris S, Kirkcaldy R, et al. Genomic epidemiology of gonococcal resistance to extended-spectrum cephalosporins, macrolides, and fluoroquinolones in the United States. J Infect Dis 2016; 214:1579–87. [PubMed: 27638945]
29. Pham CD, Sharpe S, Schlanger K, et al. Emergence of *Neisseria gonorrhoeae* strains harboring a novel combination of azithromycin-attenuating mutations. Antimicrob Agents Chemother 2019; 63:e02313–18. [PubMed: 30917979]
30. Fifer H, Cole M, Hughes G, et al. Sustained transmission of high-level azithromycin-resistant *Neisseria gonorrhoeae* in England: an observational study. Lancet Infect Dis 2018; 18:573–81. [PubMed: 29523496]
31. DaSilva D, Peters J, Cole K, et al. Whole-genome sequencing to determine transmission of *Neisseria gonorrhoeae*: an observational study. Lancet Infect Dis 2016; 16:1295–303. [PubMed: 27427203]
32. Williamson D, Chow E, Gorrie C, et al. Bridging of *Neisseria gonorrhoeae* lineages across sexual networks in the HIV pre-exposure prophylaxis era. Nat Commun 2019; 10:3988. [PubMed: 31488838]
33. Chisholm SA, Wilson J, Alexander S, et al. An outbreak of high-level azithromycin resistant *Neisseria gonorrhoeae* in England. Sex Transm Inf 2016; 92:365–7.
34. Smolarchuk C, Wensley A, Padfield S, et al. Persistence of an outbreak of gonorrhea with high-level resistance to azithromycin in England, November 2014-May 2018. Euro Surveill 2018; 23:1800287. [PubMed: 29897041]

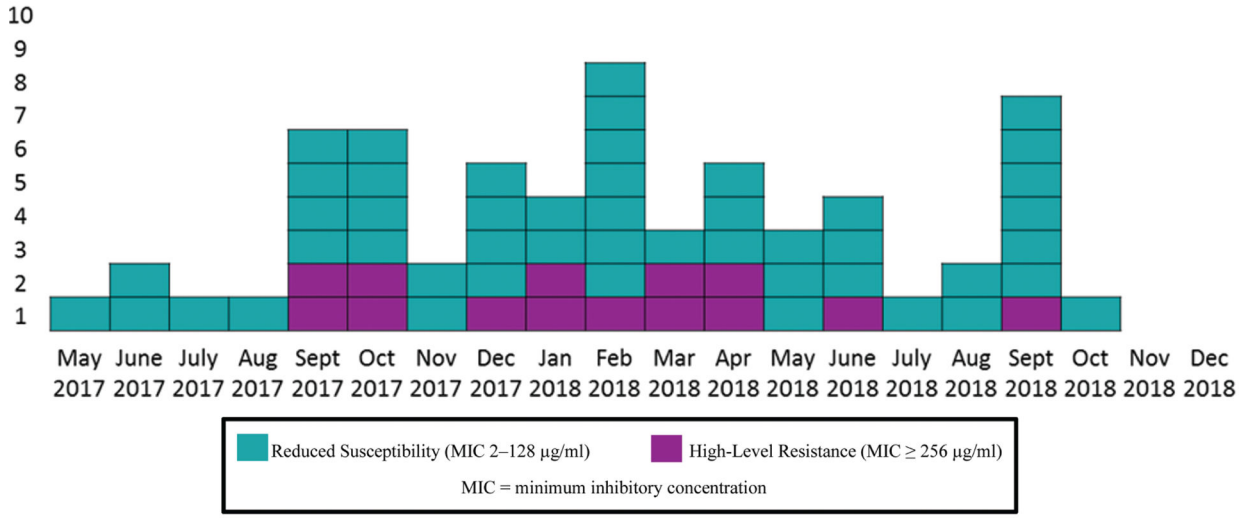

Figure 1.

Number of isolates with reduced azithromycin susceptibility or high-level azithromycin resistance each month (Indiana, from May 2017 to December 2018). Abbreviation: MIC, minimum inhibitory concentration.

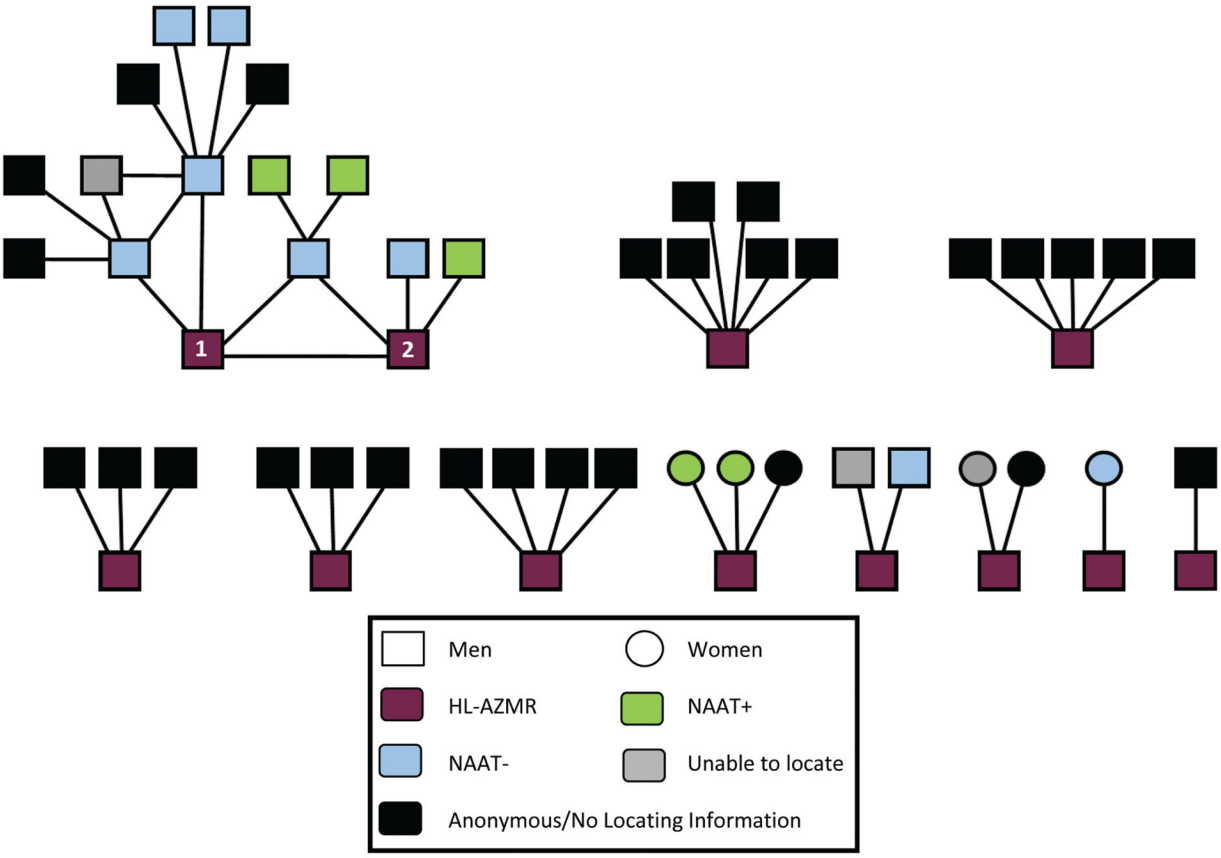

Figure 2.
Investigation outcomes of cases with high-level azithromycin resistance (HL-AZMR, high-level azithromycin resistance). Abbreviation: NAAT, nucleic acid amplification test.

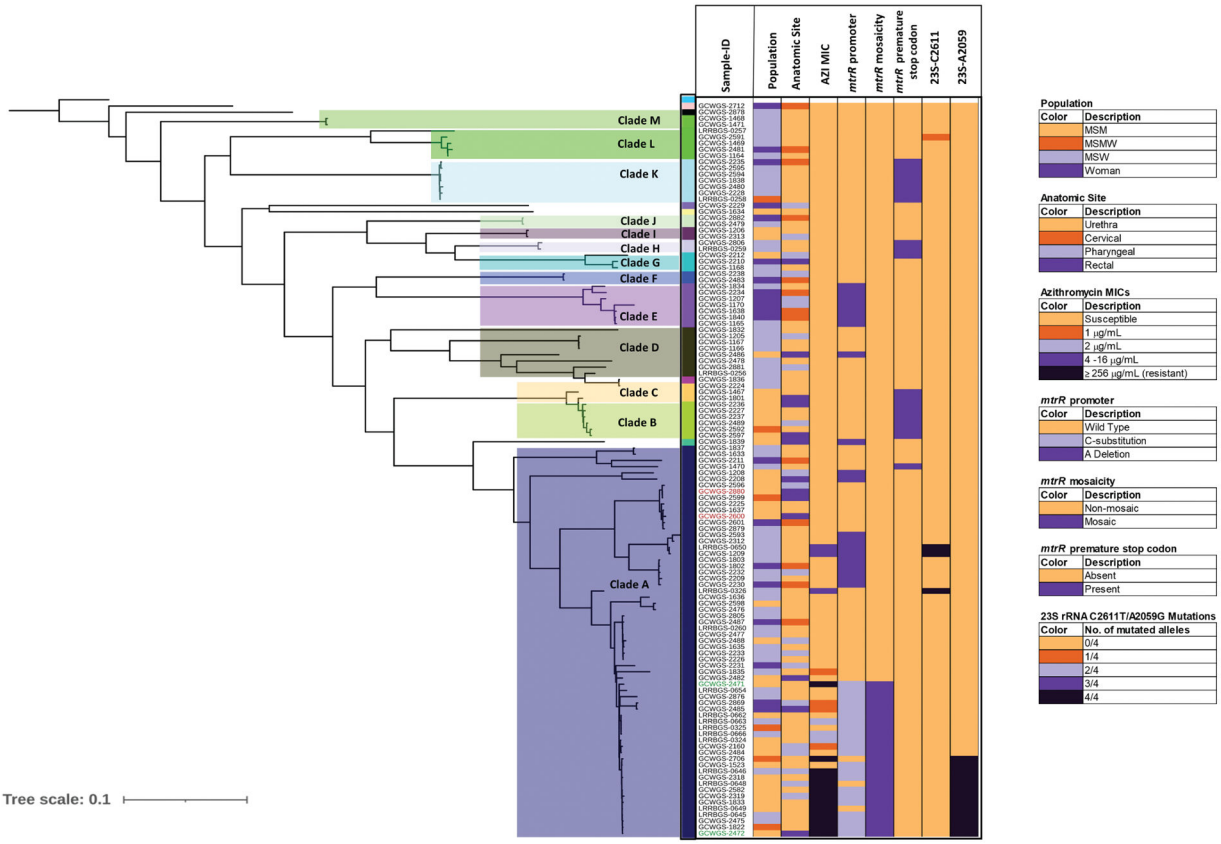

Figure 3.

Phylogenetic tree of 119 *Neisseria gonorrhoeae* isolates from Indiana, 2016–2018. Isolate sample identifiers (IDs) in red and green font denote sequences from isolates of patients who named each other as sexual partners (as identified through disease investigations). Abbreviations: AZM, azithromycin; MICs, minimum inhibitory concentrations; MSM, men who have sex only with men; MSMW, men who have sex with men and women; MSW, men who have sex only with women; rRNA, ribosomal RNA.

Table 1.

Characteristics of Patients Whose *Neisseria gonorrhoeae* Isolates Underwent Antimicrobial Susceptibility Testing, Classified by the Isolate's Azithromycin Susceptibility (Indiana, May 2017 to December 2018)

	Patients, No. (%)[a]				
Characteristics	**AZM Susceptible (n = 928)**	**AZM-RS (n = 47)**	**HL-AZMR (n = 12)**	**Total (N = 987)**	***P* Value[b]**
Sex and sexual behavior					
Male	880	44	12	936	
MSM	142 (16.1)	16 (36.4)	7 (58.3)	165 (176)	.02
MSMW	43 (4.9)	1 (2.3)	2 (16.7)	46 (4.9)	
MSW	677 (76.9)	27 (61.4)	3 (25.0)	707 (75.6)	
Unknown/missing sex of partner	18 (2.1)	0	0	18 (1.9)	
Women	48	3	0	51	…
Race/ethnicity					
Black, non-Hispanic	663 (71.5)	30 (63.8)	2 (16.7)	695 (70.4)	.004
White, non-Hispanic	206 (22.2)	15 (31.9)	9 (75)	230 (23.3)	
Hispanic	43 (4.6)	1 (2.1)	0	44 (4.5)	
Other	16 (1.6)	1 (2.1)	1 (8.3)	18 (1.8)	
Age, median (IQR), y	28 (25−35)	31 (23−38)	28 (24−40)	28 (23−35)	.74
HIV infection at time of visit					
Yes	21 (2.3)	1 (2.1)	0	22 (2.2)	…
No	636 (68.7)	46 (97.9)	12 (100.0)	694 (70.3)	
Unknown	271 (29.2)	0	0	271 (275)	
History of gonorrhea	251 (271)	16 (34.0)	7 (58.3)	274	.12
Use of geospatial apps to meet partner(s)[c]	…[d]	13 (27.7)	5 (41.7)	18	.35
>3 Partners[c]	…[d]	9 (19.1)	5 (41.7)	14	.10
Anonymous partner(s)[c]	…[d]	20 (42.6)	6 (50.0)	26	.64

Abbreviations: AZM, azithromycin; AZM-RS, reduced AZM susceptibility; HIV, human immunodeficiency virus; HL-AZMR, high-level AZM resistance; IQR, interquartile range; MSM, men who have sex only with men; MSMW, men who have sex with men and women; MSW, men who have sex only with women.

[a]Data represent no. (%) of patients unless otherwise specified.

[b]Statistical analysis compared AZM-RS with HL-AZMR.

[c] Sexual partner(s) within previous 60 days.

[d] This information is collected only during field investigations; investigations are not tracked or conducted for persons with susceptible isolates.