The Distribution and Spread of Susceptible and Resistant *Neisseria gonorrhoeae* Across Demographic Groups in a Major Metropolitan Center

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Background. Genomic epidemiology studies of gonorrhea in the United States have primarily focused on national surveillance for antibiotic resistance, and patterns of local transmission between demographic groups of resistant and susceptible strains are unknown.

Methods. We analyzed a convenience sample of genome sequences, antibiotic susceptibility, and patient data from 897 gonococcal isolates cultured at the New York City (NYC) Public Health Laboratory from NYC Department of Health and Mental Hygiene (DOHMH) Sexual Health Clinic (SHC) patients, primarily in 2012–2013. We reconstructed the gonococcal phylogeny, defined transmission clusters using a 10 nonrecombinant single nucleotide polymorphism threshold, tested for clustering of demographic groups, and placed NYC isolates in a global phylogenetic context.

Results. The NYC gonococcal phylogeny reflected global diversity with isolates from 22/23 of the prevalent global lineages (96%). Isolates clustered on the phylogeny by patient sexual behavior ($P < .001$) and race/ethnicity ($P < .001$). Minimum inhibitory concentrations were higher across antibiotics in isolates from men who have sex with men compared to heterosexuals ($P < .001$) and white heterosexuals compared to black heterosexuals ($P < .01$). In our dataset, all large transmission clusters (≥ 10 samples) of *N. gonorrhoeae* were susceptible to ciprofloxacin, ceftriaxone, and azithromycin, and comprised isolates from patients across demographic groups.

Conclusions. All large transmission clusters were susceptible to gonorrhea therapies, suggesting that resistance to empiric therapy was not a main driver of spread, even as risk for resistance varied across demographic groups. Further study of local transmission networks is needed to identify drivers of transmission.

Keywords. gonorrhea; whole genome sequencing; epidemiology; antimicrobial resistance; sexually transmitted infection.

Rates of reported *Neisseria gonorrhoeae* infections (gonorrhea) in the United States have fluctuated in the antibiotic era. After peaking in the early 1970s, rates dropped to a historic low in the US in 2009 [1]. However, since then, rates have climbed, with 583 405 gonorrhea cases reported in 2018, up 82.6% from 2009 [1]. The trends in reported cases of gonorrhea vary by sexual behavior and demographics. Men who have sex with men (MSM) have higher rates of gonorrhea infection than heterosexual people, and rates in the MSM population have been increasing more rapidly [1]. Men and women aged 20–24 years old are at highest risk, and there are major disparities in gonorrhea infection rates across race/ethnicity subgroups; rates among black Americans were 7.7 fold higher than rates among white Americans in 2018 [1].

N. gonorrhoeae infection has become a major public health concern [2, 3], given its increasing incidence and decreased antibiotic susceptibility [4–6]. As gonorrhea treatment is most commonly empiric, treatment recommendations in the US are informed by antibiotic susceptibility patterns of isolates cultured at public health laboratories from specimens collected at sentinel sexually transmitted disease clinics [1]. The prevalence of resistance varies by risk group and has resulted at times in population-specific empiric antibiotic treatment recommendations [7]. However, the extent to which gonorrhea spreads between demographic groups has been unclear. Understanding the patterns of spread and the factors driving change in incidence

is critical for the design of effective clinical and public health measures to reduce the overall burden of disease and slow the spread of resistance.

Genomic epidemiology studies have begun to address questions on transmission between demographic groups, as well as on resistance prevalence. Analysis of a global collection of *N. gonorrhoeae* spanning several decades revealed 2 major circulating gonococcal lineages, a lineage that is primarily associated with MSM and that tends to be multidrug resistant, and another associated with heterosexuals that tends to be more antibiotic susceptible [8]. However, studies of transmission within smaller geographic regions and focused on more recent samples demonstrated extensive bridging between MSM and heterosexual populations, suggesting limited gonococcal lineage association with subpopulations [9, 10]. Studies in the United States have focused primarily on national samples of strains resistant to antibiotics to define the genetic determinants of antibiotic resistance [11–13] or transmission of specific resistant lineages [14], and sampling may impact our understanding of gonorrhea transmission using genomic methods.

We sought to understand the local patterns of transmission of gonorrhea and antibiotic resistance across demographic groups. To do so, we sequenced and analyzed genomes from a sample of *N. gonorrhoeae* isolates cultured at the New York City (NYC) Public Health Laboratory (PHL) from diagnostic specimens collected from individuals attending Sexual Health Clinics (SHCs) run by the NYC Department of Health and Mental Hygiene (DOHMH). We used genomic data to describe transmission of antibiotic resistant and sensitive gonorrhea in NYC, and, using detailed patient demographic and clinical information linked to the isolates, we examined the relationship between patient groups, gonorrhea transmission, and antibiotic resistance.

METHODS

Sample Collection

The retrospective convenience sample of 897 isolates of *N. gonorrhoeae* were cultured by NYC PHL from specimens collected from 822 patients who visited the NYC DOHMH SHCs between July 2011 and September 2015. The majority of isolates (98.4%) were collected between January 2012 and June 2014. The isolates were collected as part of NYC's standard contribution of urethral isolates to the Gonococcal Isolate Surveillance Project (GISP), surveillance of urethral, rectal, and pharyngeal isolates from MSM as part of the Extragenital Gonorrhea Screening (EGGS) project, and routine culture for clinical care for example, test of cure after treatment with a nonrecommended regimen, persistent symptoms, or indications for anorectal or oropharyngeal testing prior to the availability of anorectal and oropharyngeal nucleic acid amplification test (NAAT) in SHCs. For isolates collected between January

2012 and June 2014, 72% of isolates (633/881) were collected for surveillance (281 for GISP, 352 for EGGS). The remaining 28% (248/881) were collected for routine clinical care. Isolates were included in the study if viable cultures were available from NYC PHL; 281/489 isolates collected for GISP were included, and 352/665 isolates collected for EGGS were included.

Demographic Data Collection

The following SHC visit-level data on patients whose isolates were included in the sample were extracted from SHC electronic medical records: gender, age, race/ethnicity, gender of sex partners, anatomic site of specimen collection, and HIV status (Supplementary Table 1).

Representativeness of Sampled Isolates

Our sample was derived from cultured isolates stored by the NYC PHL. Using χ^2 tests, we compared the characteristics (race/ethnicity, age, gender, sex of partners) of patients with study isolates to those of patients with gonorrhea detected by NAAT-only at NYC SHCs during the same time period. Patients contributing isolates to the study were excluded from the NAAT-positive comparison group. We considered patients with gonorrhea detected at multiple anatomic sites on the same day to have a single gonorrhea diagnosis; a patient could be counted more than once if they were diagnosed with gonorrhea more than 30 days after an earlier gonorrhea diagnosis.

Isolate Growth, DNA Extraction, and Whole Genome Sequencing

Previously stored *N. gonorrhoeae* isolates were subcultured at the NYC PHL using Chocolate II Agar plates (BBL) in a humidified 5% CO_2 incubator at 35 °C. DNA was extracted from fresh overnight subculture from single colonies using QIAcube with QIAamp DNA Mini Kit (Qiagen), and the quality of extracted DNA was checked with NanoDrop (Thermo Scientific). Whole genome sequencing (WGS) was performed on the Illumina HiSeq using standard protocols at the Wellcome Sanger Institute.

Antibiotic Susceptibility Testing

Azithromycin (AZM), cefixime (CFM), ceftriaxone (CRO), and ciprofloxacin (CIP) E-strips (bioMérieux) were used to determine minimum inhibitory concentrations (MIC) at NYC PHL at the time that the initial clinical culture was performed. The CDC alert values for reduced susceptibility to these antibiotics are AZM ≥ 2 μg/mL, CFM ≥ 0.25 μg/mL, CRO ≥ 0.125 μg/mL; only ciprofloxacin has a defined resistance breakpoint per CLSI, which is CIP ≥ 1 μg/mL [1, 15, 16].

Quality Control of Genomic Data

FastQC [17] was used to assess the quality of WGS reads. Samples were removed if quality scores were poor

across reads or if the total number of reads was not sufficient to cover the expected size of the *N. gonorrhoeae* genome (~2 Mb). Metaphlan 2.5.0 [18] was used to identify sequences of nongonococcal origin, which were removed from the dataset.

Assembly

Spades 3.12 [19] was used for de novo assembly. Assemblies were corrected using the careful flag, and contigs with less than 10X coverage or 500 nucleotides in length were filtered. Assemblies were annotated with Prokka 1.13 [20]. Additionally, reads from all isolates were mapped to NCCP11945 (NC_011035.1) [21] using the alignment algorithm BWA-MEM [22]. Pilon v 1.16 was used to identify variants with a minimum depth of 10 reads and minimum mapping quality of 20 [23]. Pseudogenomes were created by incorporating single nucleotide polymorphisms (SNPs), small deletions, and uncertain positions into the reference genome.

Phylogenetic Analysis and Clustering

We used Gubbins [24] to identify and mask recombinant regions and RAxML [25] to estimate the phylogeny of our sample. The phylogeny was based on 27112 nonrecombinant SNPs; the total number of SNPs from the unmasked alignment was 63338. We used fastbaps [26] to partition the phylogeny into BAPS groups. To identify transmission clusters within our sample, we grouped isolates based on nonrecombinant SNP differences (Supplementary Material, Supplementary Figure 1).

Visualizations

Phylogenies were visualized with ITOL [27]. Plots were made with ggplot [28], ggpubr (https://rpkgs.datanovia.com/ggpubr/), and cowplot (https://github.com/wilkelab/cowplot).

Statistics

Fritz and Purvis D [29], implemented in caper (https://cran.r-project.org/web/packages/caper/index.html), was used to test for phylogenetic structure of discrete traits. Fisher's Exact Test or χ^2 test was used to test for associations between categorical variables. The Kruskal Wallis test was used to test for associations between MICs and patient demographics, and the Wilcoxon rank sum test was used to test the significance of pairwise comparisons.

Data Availability

WGS data were deposited in the European Nucleotide Archive (ERA) under study accession PRJEB10016 (Supplementary Table 1). All additional data and scripts are available at https://github.com/gradlab/GC_NYC.

The NYC DOHMH considered this project to be public health surveillance that does not meet the Office of Human Research Protections definition of human subjects research.

RESULTS

In our convenience sample of isolates, most were cultured from specimens collected from male patients (95.2%, 854/897), and of those, 73.1% (613/854) were isolated from specimens collected from men who have sex with men (MSM; Table 1). Isolates cultured from specimens from heterosexuals were primarily collected from black patients (81.4%, 180/221), whereas those from MSM were more distributed among races/ethnicities (40.0%, 245/613 Non-Hispanic (NH)-White, 27.2%, 167/613 NH-Black, 23.5%, 144/613 Hispanic, 4.7%, 29/613 NH-Asian, and 4.6%, 28/613 NH-Other). *Neisseria gonorrhoeae* was isolated from urethral (82.0%, 700/854), rectal (14.8%, 126/854),

Table 1. Patient Demographics Associated with *Neisseria gonorrhoeae* Isolates from People Attending New York City Sexual Health Clinics, 2011–2015

Patient Characteristic	Isolate Count	Isolate Percentage
Gender		
Male	854	95.2
Female	37	4.1
Transgender	6	0.7
Age, y		
<20	65	7.2
20–24	257	28.7
25–29	275	30.7
30–34	144	16.1
35–39	58	6.5
40–44	45	5.0
45–49	22	2.5
50+	31	3.5
Sexual Behavior Groups		
MSM	613	68.3
MSW	186	20.7
MSMW	40	4.5
WSM	35	3.9
WSMW	2	0.2
TSM	6	0.6
Unknown	15	1.7
Race/Ethnicity		
NH-Black	373	41.6
NH-White	272	30.3
Hispanic	181	20.2
NH-Asian	29	2.2
NH-Other	42	4.7
HIV Status		
Positive	131	14.6
Recent Negative	675	75.3
Unknown	91	10.1
Site of Specimen Collection		
Urethral	701	78.1
Anal	130	14.5
Cervical	34	3.8
Oral	32	3.6

HIV, Human Immunodeficiency Virus; MSM, Men who have sex with men; MSMW, Men who have sex with men and women; WSM, Women who have sex with men; WSMW, Women who have sex with men and women; TSM, transgender persons who have sex with men; NH, Non-Hispanic.

and pharyngeal (3.3%, 28/854) specimens in men. In women, *N. gonorrhoeae* was isolated from cervical (92.0%, 34/37), pharyngeal (5.4%, 2/37), and rectal (2.7%, 1/37) specimens.

During the study period, 59 420 gonorrhea infections in 48 648 people living in NYC were reported to the DOHMH, so our convenience sample represents 1.5% (897/59 420) of total infections in NYC. Eighteen percent of gonorrhea infections in NYC and 23% of infections among men in NYC are in patients seen at the DOHMH SHCs. The vast majority of *N. gonorrhoeae* infections diagnosed in the SHCs were detected by NAAT during the period our isolates were collected (9740 infections excluding patients contributing to study isolates). People less than 20 years old, heterosexuals, and non-white MSM were underrepresented in the population contributing study isolates as compared to the population with NAAT-positive events at the DOHMH SHC (Supplementary Table 2).

For 33 people, *N. gonorrhoeae* was isolated from specimens collected from more than one anatomic site at the same visit. In 18.2% (6/33) of these patients, the isolates from different anatomic sites were distinct strains, which we defined as isolates with greater than 10 nonrecombinant SNP differences (Supplementary Figure 2A). We also observed 87 isolates from 41 people who returned to the SHCs multiple times; 90.5% (38/42) of re-infections were with a new strain. Re-infection with the same strain occurred up to 223 days after the original collection date (Supplementary Figure 2B). For 2 pairs of isolates, 1 from specimens collected at the same visit and 1 from specimens collected at multiple visits, the SNP distances were just outside our threshold for considering the isolates the same strain (12 and 15 SNPs). One isolate from each pair has unique recombination events, so SNP distances in these pairs may be inflated by unidentified recombination.

Neisseria gonorrhoeae Population Structure
We reconstructed the population structure of our NYC sample to examine the relationship between the gonococcus population and patient demographic groups. In a maximum likelihood phylogeny based on the nonrecombinant portions of the genome, we observed 2 main lineages of *N. gonorrhoeae* (Figure 1A). Lineage A was significantly associated with MSM isolates, and lineage B was significantly associated with isolates from heterosexual patients ($P < 2.2 \times 10^{-16}$, Figure 1B). The phylogeny was significantly structured by sexual behavior ($P < .001$) and race/ethnicity ($P < .001$).

To test whether the gonococcal population in NYC represented a subset of globally circulating *N. gonorrhoeae*, we compiled a dataset of publicly available *N. gonorrhoeae* genomes [30] (Supplementary Table 3) and described the population structure using fastbaps [26]. The genome of at least one NYC isolate was present in 72% (33/46) of BAPS groups. We defined common BAPS groups as those containing at least 1% of the total genomes in the global dataset. A NYC genome was present in 96% (22/23) of these common groups.

Characteristics of Transmission Clusters
We found that 65% (581/897) of isolates were clustered with at least one other isolate in our sample and identified 10 clusters with at least 10 isolates per cluster (Figure 1A, C). Using patient metadata, we characterized the demographic variation among the largest clusters (≥10 isolates) in our dataset (Figure 1C). Seven of 10 clusters contained isolates from both MSM and heterosexuals, suggesting bridging across sexual networks. The individuals with gonococcal isolates in these large clusters also represented multiple races/ethnicities and varied age. The distribution of HIV status across the phylogeny was not significantly different from random ($P = .28$), and the transmission clusters included HIV positive and negative patients (Figure 1C).

Characteristics of Unclustered Isolates
We compared the demographic characteristics of clustered and unclustered isolates to determine whether unsampled transmission was associated with particular patient groups. MSM-associated isolates were more likely to be clustered than isolates from heterosexual patients ($P = 5.0 \times 10^{-4}$, Figure 2A, B). We examined the association between race/ethnicity and clustering of isolates separately for each sexual behavior group. We found no association between race/ethnicity and clustering among genomes from isolates from MSM ($P = .518$). However, among heterosexuals, race/ethnicity was associated with clustering ($P = .046$, Figure 3A); genomes from isolates from white patients were more likely to be unclustered (Figure 3B).
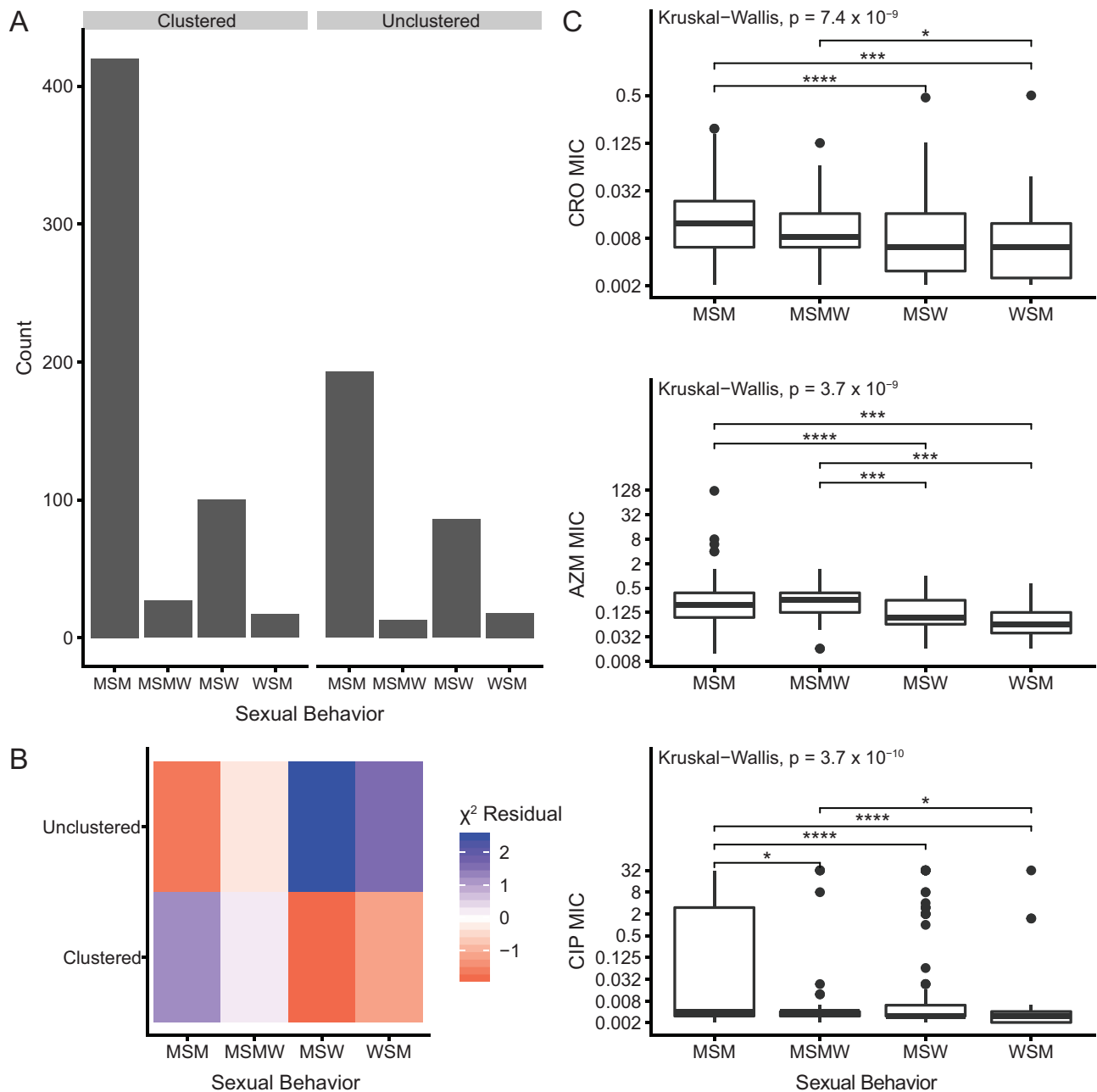
Antibiotic Susceptibility
Among isolates in our sample that underwent antibiotic susceptibility testing at NYC PHL, 24.3% (216/889) were resistant to ciprofloxacin, 0.9% (8/889) had reduced susceptibility to azithromycin, 0.3% (3/887) had reduced susceptibility to ceftriaxone, and 0.1% (1/886) had reduced susceptibility to cefixime. No isolates had reduced susceptibility to both ceftriaxone and azithromycin. The largest clusters were susceptible to currently recommended empiric therapy (ceftriaxone and azithromycin) as well as cefixime and ciprofloxacin (Figure 4). Within clusters, we observed isolates with a wide range of MICs. For some clusters (27, 79, 2, 5), this range is attributable to a 2 bp deletion disrupting the reading frame of *mtrC*, a component of the Mtr efflux pump in a subset of isolates.

As defining the patterns of the spread of isolates exhibiting antibiotic resistance may aid in surveillance and intervention strategies, we examined associations between MICs and patient demographics. Isolates from MSM had significantly higher mean MICs than isolates from heterosexuals (Figure 2C). However, no significant differences persist after controlling
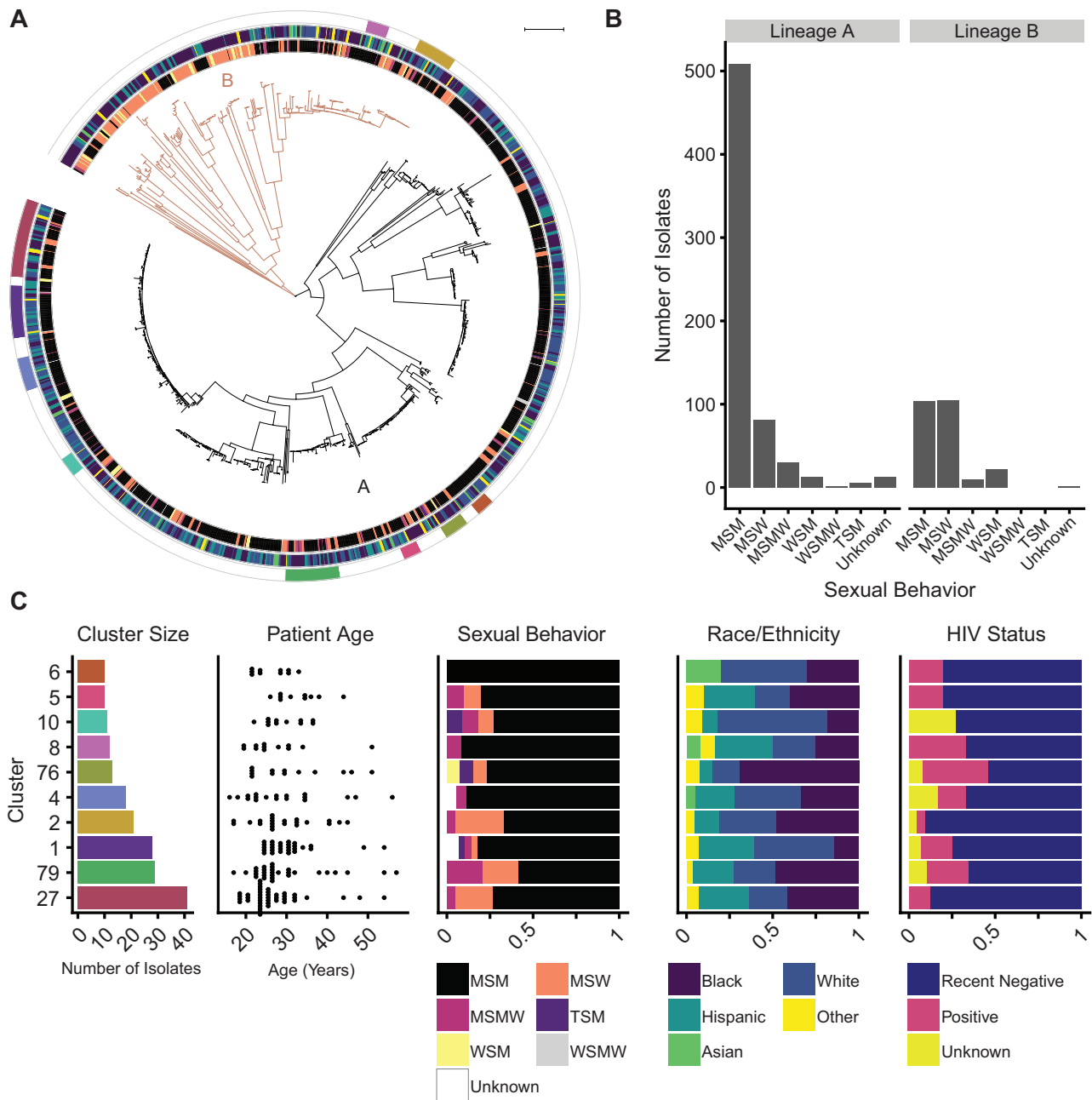
Figure 1. Population structure and transmission clusters of *Neisseria gonorrhoeae* in New York City. *A*, *N. gonorrhoeae* phylogeny is structured by sexual behavior groups, defined by patient gender and the gender of their sex partners, and race/ethnicity. The maximum likelihood phylogeny was estimated from a pseudogenome alignment with recombinant regions masked. The phylogeny can be divided into two main lineages, lineage A (black) and lineage B (orange). The annotation rings represent sexual behavior groups, race/ethnicity, and transmission clusters with >=10 isolates from the innermost to outermost rings. For the sexual behavior groups, black is men who have sex with men (MSM), orange is men who have sex with women (MSW), pink is men who sex with men and women (MSMW), yellow is women who have sex with women (WSW), gray is women who have sex with men and women (WSMW), purple is transgender persons who have sex with men (TSM), and white is unknown sexual behavior group. For the race/ethnicity annotation, purple is black, blue is white, teal is Hispanic, green is Asian, and yellow is other. *B*, Lineage A is associated with MSM, and lineage B is associated with heterosexual patients. We found a significant association between the major lineages and sexual behavior group ($P < 2.2 \times 10^{-16}$). *C*, Strains comprising largest transmission clusters are transmitting across demographic groups. Transmission connections were identified using a 10 nonrecombinant SNP cutoff, and transmission clusters were defined as clusters of isolates connected to at least one other member of the cluster and any additional isolates nested within the phylogeny. Using this method, we identified 10 clusters with at least 10 isolates (leftmost graph, colors match annotation ring in panel (*A*). Strains are transmitting across age groups; each point in the age graph (second to left) is the age of a patient associated with an isolate in the cluster. Strains are also transmitting across MSM and heterosexual networks and multiple races/ethnicities (third and fourth to left); color blocks correspond to the proportion of isolates within the cluster associated with each group (legend below graph). Abbreviation: HIV, human immunodeficiency disease.
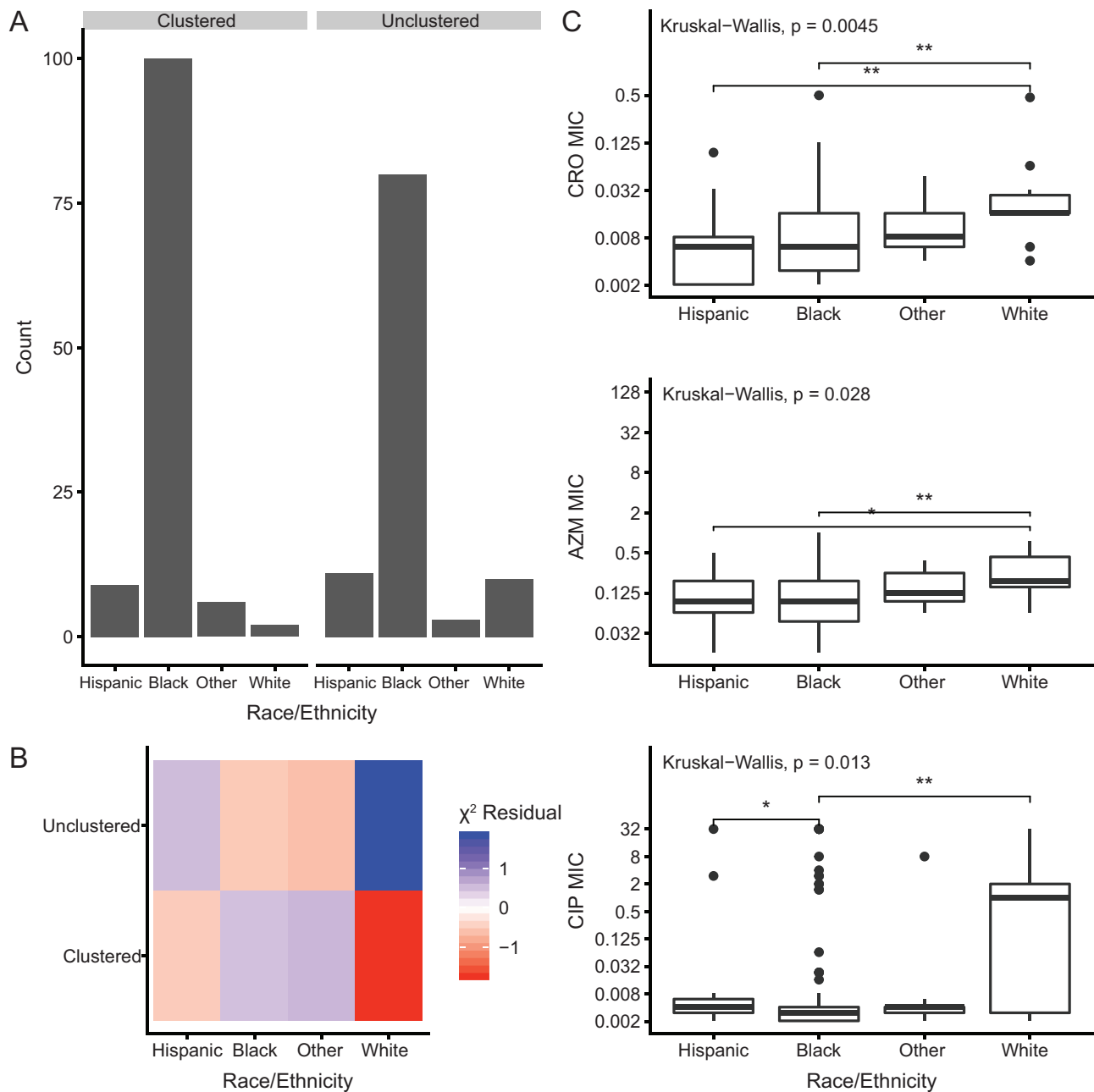
Figure 2. Sexual behavior groups are associated with clustering and antibiotic susceptibility. *A,* Sexual behavior groups and clustering are associated. Isolates were considered clustered if they were grouped with at least one other isolate in the dataset using the 10 nonrecombinant SNP cutoff. We identified a significant association between sexual behavior groups and whether or not an isolate was clustered ($P = 5.0 \times 10^{-4}$). *B,* Clustered isolates are associated with MSM, and unclustered isolates are associated with heterosexuals. The χ^2 residual for each category is displayed where blue represents more isolates than expected for the category and red represents fewer isolates than expected for the category. *C,* MSM-associated isolates have higher MICs across antibiotics (including cefixime, $P = 3.3 \times 10^{-5}$). Significant pairwise comparisons are denoted by a bracket and asterisks (* $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$). Abbreviations: AZM, azithromycin; CIP, ciprofloxacin; CRO, ceftriaxone; MIC, minimum inhibitory concentrations; MSM, men who have sex with men; MSW, men who have sex with women; MSMW, men who sex with men and women; WSM, women who have sex with women.

for lineage for ceftriaxone and azithromycin; ciprofloxacin MICs are significantly higher in lineage A isolates from MSM ($P = .004$, Supplementary Figure 3).

Among isolates from MSM, we found no differences across race/ethnicity subgroups in the mean MICs of ceftriaxone and azithromycin; however, there was an association between MSM race/ethnicity and ciprofloxacin MICs ($P = .028$; Supplementary Figure 4), with greater resistance in isolates from white MSM. In heterosexuals, we found significant associations between race/ethnicity and

Figure 3. Race/ethnicity is associated with clustering and antibiotic susceptibility among heterosexuals. *A*, Race/ethnicity of heterosexuals and clustering are associated. Isolates were considered clustered if they were grouped with at least one other isolate in the dataset using the 10 nonrecombinant SNP cutoff. We identified a significant association between heterosexual race/ethnicity and whether an isolate was clustered ($P = .04563$). *B*, Unclustered isolates are associated with white heterosexuals. The χ^2 residual for each category is displayed where blue represents more isolates than expected for the category and red represents fewer isolates than expected for the category. *C*, Isolates from white heterosexuals have higher MICs than isolates from black heterosexuals across antibiotics, including cefixime ($P < .01$). Significant pairwise comparisons are denoted by a bracket and asterisks (* $P < .05$, ** $P < .01$). Abbreviations: AZM, azithromycin; CIP, ciprofloxacin; CRO, ceftriaxone; MIC, minimum inhibitory concentrations.

mean MICs for ceftriaxone, azithromycin, and ciprofloxacin (Figure 3C), with MICs for isolates from white heterosexual patients significantly higher than in isolates from black heterosexual patients (Figure 3C). These differences may also be attributable to lineage: of the 12 isolates from white heterosexual patients, only one was in lineage B.

DISCUSSION

We incorporated the analysis of phenotypic susceptibility results, patient demographics, and gonococcal genome sequences to investigate transmission in a major metropolitan area. We found that the NYC population contains representatives from most major groups in a global sample. We also found that *N. gonorrhoeae*
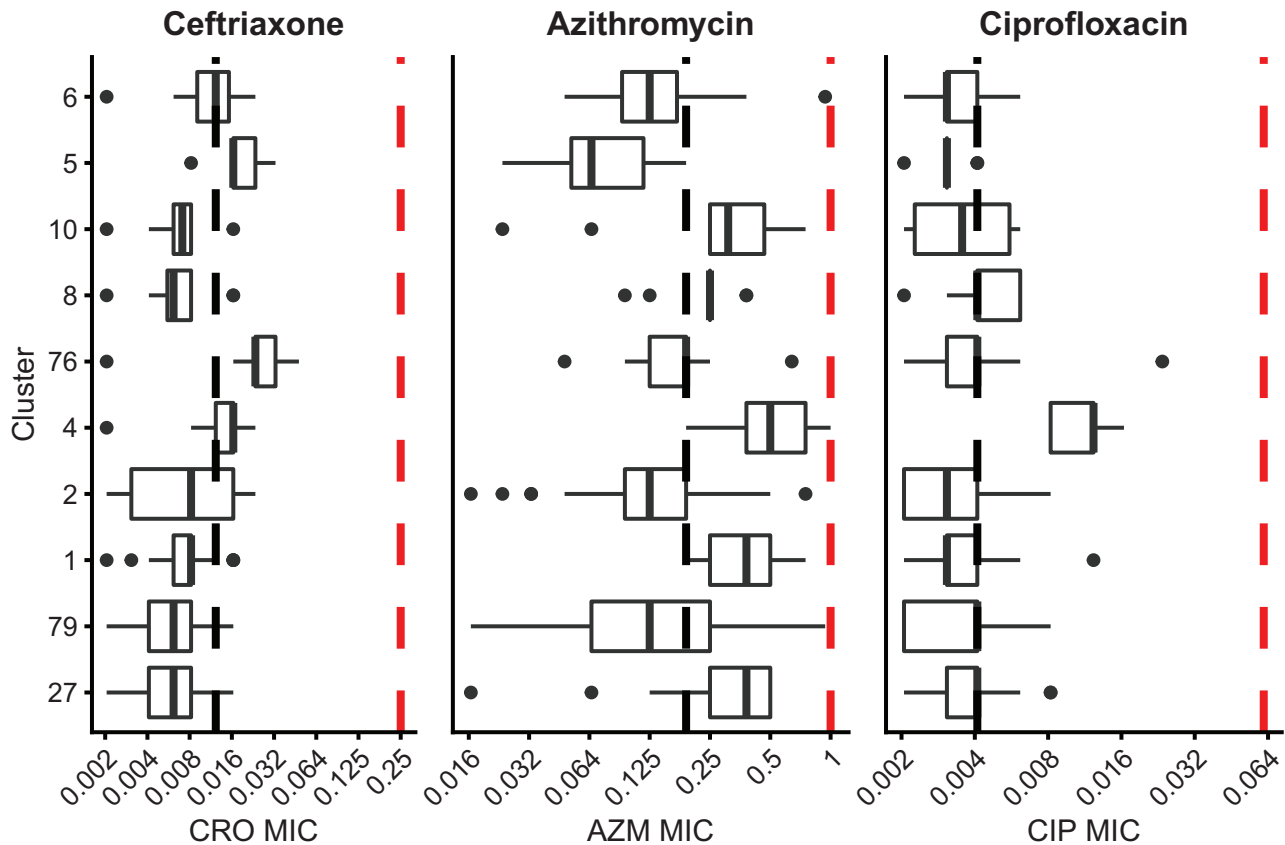
Figure 4. Isolates from large transmission clusters are sensitive to antibiotics. Minimum inhibitory concentrations (MIC, μg/mL) to ceftriaxone (CRO), azithromycin (AZM), and ciprofloxacin (CIP) for each isolate were measured using E-test. Cluster numbers correspond to Figure 1C. Black dashed lines represent median MICs for the dataset. All isolates have MICs below the susceptibility breakpoint (red dashed line) for all antibiotics, including cefixime (CRO ≤ 0.25 μg/mL, CFM ≤ 0.25 μg/mL, AZM ≤ 1 μg/mL, CIP ≤ 0.06 μg/mL). Isolates in clusters do not have uniformly elevated MICs compared to the overall distribution, suggesting that antibiotic resistance was not the main driver of gonorrhea transmission in NYC during this time period. Abbreviation: NYC, New York city.

lineages were associated with sexual behavior groups, and we found a significant association between antibiotic susceptibility and patient demographic groups. We identified transmission clusters and found that the largest clusters were associated with patients from multiple demographic groups and consisted of *N. gonorrhoeae* isolates susceptible to gonorrhea therapy.

Our dataset comprised a retrospective sample of isolates derived from specimens collected from patients presenting at NYC SHCs for surveillance, diagnosis, and treatment of gonorrhea. Other providers (private providers, family planning, and others) make most of the reported gonorrhea diagnoses in NYC (82%), and our results show that not all patient populations are equally likely to be sampled for culture and susceptibility testing at SHCs. A large portion of NYC isolates (~35%) in our dataset were not clustered with any other isolate, suggesting that these transmission networks were not well sampled. Transmission links may have been missed in the setting of mixed infection because we sequenced single colony isolates. Isolates from MSM were more likely to be clustered than isolates from heterosexuals, and among heterosexuals, isolates from non-white

patients were more likely to be clustered. This is likely related to sampling at SHCs, both because of the populations that attend SHCs [31] and the criteria for sending a specimen for culture, but may also be due to differences in sexual behavior.

Despite the convenience sampling, several findings emerge from our study. We found that almost all major clades from a global collection of *N. gonorrhoeae* genomes were represented in the NYC sample, highlighting the frequent transmission between regions and lack of geographic structure, consistent with other studies [8, 9, 32, 33]. The phylogeny of the NYC *N. gonorrhoeae* population can be divided into the previously described 2 major global lineages, lineages A and B. A previous study of a global collection found a significant association between isolates from female patients and lineage B, driving a hypothesis that isolates from lineage B are primarily found in heterosexuals and isolates from lineage A in MSM [8]. In our dataset, we confirmed these associations between lineage and sexual behavior groups in the setting of a metropolitan area. However, our data also suggested bridging between sexual behavior groups in the largest transmission clusters. One possible

explanation for this seemingly contradictory result is that frequent bridging is a recent phenomenon, and the phylogenetic structure is the result of historical sexual networks. Another possibility is that while frequent bridging occurs, lineage A isolates are more successful in MSM networks and lineage B isolates are more successful in heterosexual networks. Further studies are needed to understand the relationship between *N. gonorrhoeae* population structure and adaptation to different sexual behavior groups.

Studies analyzing HIV status in the context of gonorrhea transmission networks in other regions have observed a lack of serosorting among HIV positive and HIV negative individuals [10, 34, 35]. As HIV pre-exposure prophylaxis (PrEP) was uncommon during 2012–2013 [36, 37], our observations of gonorrhea transmission clusters with both HIV positive and negative patients suggest that in the sample population from NYC effective serosorting was not practiced in the era prior to PrEP introduction. Our results support CDC guidelines that a diagnosis with gonorrhea is an indication for PrEP [38].

Isolates from MSM had higher mean MICs across antibiotics compared to isolates from heterosexuals, primarily due to differences in susceptibility in lineages A and B. Data from national surveillance (GISP) also showed an association between MSM-associated isolates and reduced susceptibility to antibiotics: in 2018, 8.2% of isolates from MSM had azithromycin MICs $\geq$2.0 μg/mL compared to 2.4% of isolates from MSW, and 0.22% of isolates from MSM had ceftriaxone MICs $\geq$0.125 μg/mL compared to 0.16% of isolates from MSW [1]. Resistant *N. gonorrhoeae* strains may spread faster in MSM populations compared to heterosexual populations because of higher treatment rates among MSM [39].

We also found that among heterosexuals, isolates from white patients had higher MICs than isolates from other races/ethnicities, and the majority of isolates from white heterosexual patients belonged to lineage A. This may reflect differences in overall antibiotic use, diagnosis, or treatment in these populations. A surveillance study of quinolone resistant *N. gonorrhoeae* in southern California in 2001–2002 found that non-white patients were at lower risk for quinolone-resistant gonorrhea [40]. There is a relationship between seasonal azithromycin use and resistance in *N. gonorrhoeae,* suggesting a role for bystander selection (selection for resistance in bacteria other than the intended target of therapy) in the development and maintenance of resistance in the *N. gonorrhoeae* population [41]. Given that white Americans may consume twice the antibiotic prescriptions compared to other race/ethnicities [42, 43], *N. gonorrhoeae* infecting white Americans may be subject to increased bystander selection. More studies on the relationship between antibiotic use across demographic groups and their risk for resistant gonorrhea are needed.

In the United States, genomics has been primarily used to understand the biology and transmission of antibiotic-resistant gonococcus. However, the major transmission clusters in our study involved strains that were susceptible to currently recommended empiric therapy, as well as cefixime and ciprofloxacin, indicating that during the study period, antibiotic resistance was not a major driver of gonorrhea transmission in NYC. This dataset was collected primarily in 2012–2013, and resistance to azithromycin may have contributed to more recent gonorrhea outbreaks [13], as azithromycin reduced susceptibility has increased from 0.6% to 4.6% from 2013 to 2018 [1]. Within clusters, we observed a wide range of MICs. In some clusters, all with evidence of bridging, the range of MICs could be attributed to a portion of isolates encoding a 2bp deletion in *mtrC*, which is associated with antibiotic susceptibility and the cervical niche [30]. While resistance remains a major public health concern, strategies to reduce overall gonorrhea transmission are also needed as pre-existing transmission networks may present opportunities for rapid spread of resistant lineages. Additionally, fewer cases overall allows public health programs to concentrate more resources on resistant cases. Greater understanding of the transmission dynamics of both susceptible and resistant infections can aid the design of effective intervention strategies for controlling gonorrhea, and further investment in sexual health services and interventions are critical.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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