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Permalink

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Journal

The Journal of Infectious Diseases, 227(7)

ISSN

0022-1899

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Publication Date

2023-04-12

DOI

10.1093/infdis/jiad027

Peer reviewed

Whole-Genome Sequencing to Predict Antimicrobial Susceptibility Profiles in *Neisseria gonorrhoeae*

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Background. *Neisseria gonorrhoeae* is a major public health problem due to increasing incidence and antimicrobial resistance. Genetic markers of reduced susceptibility have been identified; the extent to which those are representative of global antimicrobial resistance is unknown. We evaluated the performance of whole-genome sequencing (WGS) used to predict susceptibility to ciprofloxacin and other antimicrobials using a global collection of *N. gonorrhoeae* isolates.

Methods. Susceptibility testing of common antimicrobials and the recently developed zolifodacin was performed using agar dilution to determine minimum inhibitory concentrations (MICs). We identified resistance alleles at loci known to contribute to antimicrobial resistance in *N. gonorrhoeae* from WGS data. We tested the ability of each locus to predict antimicrobial susceptibility.

Results. A total of 481 *N. gonorrhoeae* isolates, collected between 2004 and 2019 and making up 457 unique genomes, were sourced from 5 countries. All isolates with demonstrated susceptibility to ciprofloxacin (MIC ≤0.06 μg/mL) had a wild-type *gyrA* codon 91. Multilocus approaches were needed to predict susceptibility to other antimicrobials. All isolates were susceptible to zoliflodacin, defined by an MIC ≤0.25 μg/mL.

Conclusions. Single marker prediction can be used to inform ciprofloxacin treatment of *N. gonorrhoeae* infection. A combination of molecular markers may be needed to determine susceptibility for other antimicrobials.

Keywords. *Neisseria gonorrhoeae*; antimicrobial susceptibility testing; whole-genome sequencing.

Neisseria gonorrhoeae infection has become a major threat to public health owing to increasing incidence and antimicrobial resistance [1–5]. Globally, there are >87 million cases of *N. gonorrhoeae* every year [6]. Resistance has emerged to each antimicrobial used to treat *N. gonorrhoeae*, and World Health Organization (WHO) guidelines suggest that empiric therapy should be discontinued when the prevalence of antimicrobial resistance reaches 5% of the *N. gonorrhoeae* population [2, 7]. Because of the emergence and expansion of *N. gonorrhoeae* lineages resistant to previously recommended therapies,

treatment with ceftriaxone (an injectable extended-spectrum cephalosporin) is the currently recommended therapy for *N. gonorrhoeae* infection in the United States [8]; however, cases of reduced susceptibility have increasingly been documented [3–5, 8–20]. Zoliflodacin is a promising new antimicrobial with a unique mechanism of action for the treatment of *N. gonorrhoeae* infection in a time where the antimicrobial pipeline is limited. A global phase III clinical trial for of zoliflodacin for the treatment of uncomplicated *N. gonorrhoeae* infection is underway (ClinicalTrials.gov NCT03959527) [21, 22].

As an alternative to empiric therapy, antibiotic prescribing for gonorrhea could be guided by antimicrobial susceptibility testing (AST). However, there are technical and resource constraints to widespread AST. Antimicrobial susceptibility is measured on a continuous scale using the minimum inhibitory concentration (MIC), and antimicrobial resistance is defined as having greater than a certain threshold MIC. Currently, *N. gonorrhoeae* AST uses phenotypic methods that require live organisms. Newer methods of AST are urgently needed.

Screening for bacterial genotypes to predict antimicrobial resistance phenotypes may be a valuable method to guide treatment [23–25]. For example, commercialized molecular tests (eg, SpeedDx ResistancePlus GC) [26] targeting *gyrA* codon 91 have been developed and marketed in Europe and Australia for the simultaneous detection of *N. gonorrhoeae* infection and prediction of ciprofloxacin susceptibility [27]. In addition, whole-genome sequencing (WGS) has advanced, and the application of this technology might aid in the assessment of drug susceptibility. Genetic markers of reduced susceptibility and resistance in *N. gonorrhoeae* have been identified for currently available antimicrobials [28–32]. However, those markers may not explain all of the resistance or reduced susceptibility identified through phenotypic methods. In addition, data sets with a global distribution of strains are limited and thus the extent to which those genetic determinants are representative of global antimicrobial resistance is unknown.

In the current study, we collected gonococcal isolates from diverse geographic locations where antimicrobial selection pressures vary and validated WGS for the prediction of antimicrobial susceptibility in *N. gonorrhoeae*. The primary outcome measures were to compare the performance of WGS and the genotype at previously reported molecular genetic markers in *N. gonorrhoeae* isolates to predict susceptibility to ciprofloxacin to a culture-based phenotypic method as a nonreference standard. The secondary outcome measures were to assess the performance of WGS with previously reported molecular genetic markers for the detection of susceptibility to ceftriaxone, cefixime, azithromycin, spectinomycin, penicillin, tetracycline, and zoliflodacin in *N. gonorrhoeae* isolates compared with a culture-based phenotypic method. Additional secondary outcome measures included calculating the fraction of reduced susceptibility that do not have previously reported genetic markers of resistance and describing the genetic relatedness between isolates across geographic areas. The results of this study will contribute to the development of diagnostic tools to guide clinical treatment for *N. gonorrhoeae* infections.

METHODS

Study Design

This study was designed to evaluate the validity of WGS to predict susceptibility to ciprofloxacin using a global collection of *N. gonorrhoeae* isolates. Additional aims were to determine the performance of WGS to predict susceptibility to ceftriaxone, cefixime, azithromycin, spectinomycin, penicillin, tetracycline and zoliflodacin in this collection of isolates. *N. gonorrhoeae* isolates from Canada, the Dominican Republic, Hong Kong, South Africa, and Vietnam were selected and underwent AST by agar dilution, and the isolates' genomes were sequenced (Table 1). Positive percent agreement (PPA), negative percent agreement (NPA), positive predictive value (PPV),

Table 1. Number of *Neisseria gonorrhoeae* Isolates and Sample Collection Years From Each Geographic Site

Site	Collection Years	Isolates, No.
Canada	2004–2017	42
South Africa	2013–2014	55
Hong Kong	2014–2018	225
Vietnam	2019	123
Dominican Republic	2018–2019	36
Total	…	481

and negative predictive value (NPV) were all calculated for susceptibility to antimicrobials by comparing the reference standard (MIC breakpoint) results (susceptible vs resistant or reduced susceptibility) and the genotype based on the presence of previously reported genetic markers of resistance, as described below.

Study Procedures/Evaluations

Isolates

A total of 481 *N. gonorrhoeae* isolates were sourced from 5 countries to create the collection analyzed in this study.

Phenotypic AST

The laboratory at the University of Washington performed confirmatory tests on all *N. gonorrhoeae* isolates, conducted multiple subcultures to obtain pure *N. gonorrhoeae* colonies from contaminated cultures, and then performed culture-based AST by the Clinical and Laboratory Standards Institute (CLSI)–recommended reference agar dilution methods to determine the MIC [33]. The panel of antimicrobial agents and ranges of concentrations for AST included penicillin (0.008–64.0 µg/mL), tetracycline (0.008–64.0 µg/mL), cefixime (0.001–2.0 µg/mL), ceftriaxone (0.001–2.0 µg/mL), ciprofloxacin (0.001–64.0 µg/mL), azithromycin (0.008–256.0 µg/mL), zoliflodacin (0.001–2.0 µg/mL), and spectinomycin (16.0–256.0 µg/mL).

In addition, β-lactamase production was also determined using the nitrocefin reference test [28]. For quality assurance, each AST run performed included a set of 4 well-characterized reference *N. gonorrhoeae* strains with known MICs, including American Type Culture Collection 49226 recommended by CLSI and 3 of the whole-genome sequenced 2016 WHO reference strains, WHO L, WHO O, and WHO U [28]. Reference *N. gonorrhoeae* strain AST results had to be within established MIC range for the AST run to be valid. All measured MICs for each isolate can be found in Supplementary Table 1.

WGS and Assembly

DNA was extracted from bacterial cells grown overnight on GCB-K plates at 37°C with 5% carbon dioxide using the Invitrogen PureLink Genomic DNA Mini Kit. Libraries were prepped and sequenced on the Illumina NextSeq 2000

sequencer at the Microbial Whole Genome Sequencing Center or the Bauer Core Facility at Harvard University. Read quality was assessed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were mapped to the NCCP11945 (NC_011035.1) reference genome using BWA-MEM software (version 0.7.17) [34], mapped reads were sorted and indexed with Samtools (version 1.9) [35], duplicates were marked with Picard tools (version 2.20.1) (https://broadinstitute.github.io/picard/), and variants were called using Pilon software (version 1.23) [36]. Variants were called using a minimum mapping quality of 20 and a minimum coverage of 10×.

Reads were also mapped to single copies of the 23S and 16S ribosomal DNA (rDNA) sequences using the same procedure to call variants. The quality and coverage of mapped reads were assessed using Qualimap software, version 2.2.1 [37]. We created pseudogenomes by editing the reference genome with variants supported by ≥90% of mapped reads and replacing ambiguous bases with "N" and deletions with "-". We performed de novo genome assembly with SPAdes software (version 3.12.0) [38] and filtered contigs <500 nucleotides in length or with <10× coverage. Sequencing reads have been deposited at the National Center for Biotechnology Information (BioProject accession no. PRJNA776899).

Resistance Allele Identification

Using the WGS data, we identified resistance alleles at all loci known to contribute to antimicrobial resistance in *N. gonorrhoeae*. The full list of molecular markers that modulate antibiotic susceptibility assessed is included in Table 2. The presence of single-nucleotide polymorphisms associated with resistance was determined from variant calls after mapping to the reference genome. If coverage, mapping quality, or allele frequency did not meet the thresholds described above, the genotype was not defined.

Variants in 16S and 23S rDNA were separately called based on mapping to these loci; the copy number of 23S rDNA variants was determined based on allele frequency, as described elsewhere [39]. The *penA* alleles were typed according to the NGSTAR database (last accessed 2 March 2021) from assemblies. Resistance-associated insertions and deletions were identified from assemblies. For *mtrCDE, mtrR,* and the *mtr* promoter, mosaic alleles were defined as those with <95% identity to the wild-type *mtr* locus from FA1090 (NC_002946.2). When ≥1 of *mtrCDE* in addition to the promoter region showed evidence of mosaicism, the *mtr* operon was designated as mosaic [40]. The presence of *tetM*, *bla*$_{TEM}$, and *erm* genes was determined using blastn software (version 2.9.0) [41] from assemblies using the following accessions as the query: MG874353.1, NG_068038.1, EU048318.1, and M14730.1.

Phylogenetic Analysis
An alignment of pseudogenomes was used for phylogenetic analysis. Gubbins (version 2.4.1) [42] and RAxML (version 8.2.12) [43] software were used for recombination detection and building a recombination-corrected maximum likelihood phylogeny.

Statistical Analysis
We assessed the performance of susceptibility prediction, using genetic markers from WGS data and 4 methods: predicting susceptibility using single markers, predicting susceptibility from

Table 2. Molecular Markers That Modulate Antibiotic Susceptibility

| Antimicrobial | CLSI MIC Breakpoint, µg/mL | | Sites[a] |
	Susceptibility	Resistance	
Penicillin	≤0.06	≥2	*bla*$_{TEM}$, mosaic *mtr*, mosaic *penA*, *mtr* promoter mutations, *mtrC* loss of function, *mtrR* loss of function, *mtrR* 39, *mtrR* 45, *penA* 501, *penA* 513, *penA* 517, *penA* 542, *penA* 543, *penA* 551 *porB* 120, *porB* 121
Tetracycline	≤0.25	≥2	Mosaic *mtr*, *mtr* promoter mutations, *mtrR* loss of function, *mtrC* loss of function, *mtrR* 39, *mtrR* 45, *porB* 120, *porB* 121, *rpsJ* 57, *tetM*
Ciprofloxacin	≤0.06	≥1	*gyrA* 91, *gyrA* 95, mosaic *mtr* loci, *mtr* promoter mutations, *mtrC* loss of function, *mtrR* loss of function, *mtrR* 39, *mtrR* 45, *parC* 86, *parC* 87, *parC* 91, *porB* 120, *porB* 121
Spectinomycin	≤32	≥128	16S rDNA 1187, *rpsE* 24 *rpsE* del 27, *rpsE* 82
Azithromycin	≤1	…	23S rRNA 2059, 23S rRNA 2611, duplications in *rplV, ermB, ermC*, mosaic *mtr*, *mtr* promoter mutations, *mtrC* loss of function, *mtrR* loss of function, *mtrR* 39, *mtrR* 45, *rplD* 70
Cefixime	≤0.25	…	Mosaic *penA*, *mtr* promoter mutations, *mtrC* loss of function, *mtrR* loss of function, *mtrR* 39, *mtrR* 45, *penA* 501, *penA* 513, *penA* 517, *penA* 542, *penA* 543, *penA* 551, *porB* 120, *porB* 121, *rpoB* 157, *rpoB* 158, *rpoB* 201, *rpoD* 98, *rpoD* del 92–95
Ceftriaxone	≤0.25	…	Mosaic *penA*, *mtr* promoter mutations, *mtrC* loss of function, *mtrR* loss of function, *mtrR* 39, *mtrR* 45, *penA* 501, *penA* 513, *penA* 517, *penA* 542, *penA* 543, *penA* 551, *porB* 120, *porB* 121, *rpoB* 157, *rpoB* 158, *rpoB* 201, *rpoD* 98, *rpoD* del 92–95
Zoliflodacin	…	…	*gyrB* 429, *gyrB* 450

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; del, deletion; MIC, minimum inhibitory concentration; rDNA, ribosomal DNA; rRNA, ribosomal RNA.

[a]Numbers indicate the codon analyzed. If a specific codon is not indicated, gene, loss of function allele, or mosaicism presence was used.

the presence of susceptibility-associated allele at all loci, predicting MICs from multiple markers using multivariate linear regression, and predicting MICs using previously described equations [44]. We varied MIC breakpoints for determination of antimicrobial susceptibility: ciprofloxacin (MIC, ≤0.06 and ≤0.5 µg/mL), ceftriaxone (≤0.06, ≤0.125, and ≤0.25 µg/mL), cefixime (≤0.06, ≤0.125, and ≤0.25), azithromycin (≤0.5 and ≤1 µg/mL), spectinomycin (≤32 and ≤64 µg/mL), penicillin (≤0.06 and ≤1 µg/mL), tetracycline (≤0.25 and ≤1 µg/mL), zoliflodacin (≤0.25 and ≤0.5 µg/mL) to calculate measures of validity, PPVs, and NPVs. For each antimicrobial that has a designated CLSI breakpoint, we included that breakpoint in the above (CLSI MIC breakpoints for susceptibility: ciprofloxacin, ≤0.06 µg/mL; ceftriaxone, cefixime, and tetracycline, ≤0.25 µg/mL; azithromycin, ≤1 µg/mL; spectinomycin, ≤32 µg/mL; penicillin, ≤0.06 µg/mL) [45].

We estimated the PPA, NPA, PPV, and NPV of known genetic markers (Table 2) for the detection of susceptibility to each antimicrobial (ciprofloxacin, ceftriaxone, cefixime, azithromycin, spectinomycin, tetracycline, penicillin, and zoliflodacin) compared with phenotypic antimicrobial susceptibility determination by agar dilution (measures were calculated twice using each of 2 MIC breakpoints for susceptibility: ≤0.06 and ≤0.5 µg/mL). Because multiple molecular markers may contribute to antimicrobial resistance, we also conducted multivariate linear regression modeling to predict the MIC for each isolate from a combination of molecular antimicrobial reduced susceptibility determinants for each antimicrobial [44]. We used $\log_2(\text{MIC})$ as the dependent variable in the linear regression and the genetic markers for resistance (Table 2) as the independent variables in the regression model.

The weights used for each molecular marker were generated from the coefficients from the regression and we report those equations to calculate the predicted MIC. From those we calculated the predicted MIC. The predicted MICs and MICs measured by means of agar dilution AST were plotted, and R^2 was calculated for each antimicrobial. In addition, we calculated the PPV to demonstrate the variation that existed in the prediction models.

We also predicted MICs using the multivariate regression equations from Demczuk et al [44], which were trained on a set of isolates distinct from those described here. The performance of those equations was assessed by comparing predicted and measured MICs and calculating the R^2 for each antimicrobial. In addition, we calculated the PPV for susceptibility category (susceptible, nonsusceptible, resistant, etc) given the predicted MIC and the categorical cutoffs for each antibiotic.

This research was deemed nonhuman subject research by the institutional review boards at the University of California San Diego, University of Washington, and Harvard T.H. Chan School of Public Health and therefore did not require institutional review board oversight.

RESULTS

We conducted AST and WGS for 481 *N. gonorrhoeae* isolates. The sequencing reads from 1 isolate were heavily contaminated, and comparison of single-nucleotide polymorphism distances from pseudogenomes demonstrated that an additional 23 isolates were identical to another isolate in the collection. We removed 1 isolate from the identical pairs based on assembly quality, yielding a final data set of 457 genomes (Table 1). Isolates from each geographic location were diverse and interspersed on the phylogeny (Figure 1). The collection of 457 isolates represented a range of MICs for ciprofloxacin, cefixime, ceftriaxone, ciprofloxacin, penicillin, and tetracycline, with MICs in both the susceptible and resistant or reduced susceptibility ranges (Figure 2). We did not observe any isolates with decreased susceptibility or resistance to spectinomycin or zoliflodacin.

All isolates with demonstrated susceptibility to ciprofloxacin, defined by an MIC ≤0.06 µg/mL, had a serine (the wildtype allele) encoded at *gyrA* codon 91 (lower confidence limit for 95% confidence interval [LCL], 97.2%) (Figure 1). The NPA for a phenylalanine (the mutant allele) encoded at *gyrA* position 91 and reduced susceptibility to ciprofloxacin (MIC, >0.06 µg/mL) was also 100% (LCL, 99.2%). When using a higher MIC breakpoint for ciprofloxacin susceptibility determination (≤0.5 µg/mL), the PPA and NPA for the *gyrA* 91 genotype were 78.8% (LCL, 72.1%) and 100% (99.1%), respectively. However, the PPVs and NPVs for the *gyrA* 91 genotype and ciprofloxacin susceptibility determination, defined by that higher breakpoint, were high, at 100% (LCL, 97.2%) and 92.1% (89.3%), respectively. Performance characteristics for antimicrobial susceptibility determination by other single molecular markers are included in Supplementary Table 2.

For ciprofloxacin, all of the assessed molecular markers indicated susceptibility in 7 (6.7%) of the 104 isolates that were susceptible based on phenotypic determination (MIC, ≤0.06 µg/mL). In addition, 8 of 452 (1.8%) ceftriaxone-susceptible, 8 of 438 (1.8%) cefixime-susceptible, and 3 of 450 (0.7%) azithromycin-susceptible isolates had all respective molecular markers indicating susceptibility. Thus, among isolates in which all assessed molecular markers indicated susceptibility, 100% (LCL, 65.2%) were ciprofloxacin susceptible based on the phenotypic determination, 100% (68.8%) were ceftriaxone susceptible based on the phenotypic determination (MIC, ≤0.25 µg/mL), 100% (LCL, 68.8%) were cefixime susceptible based on the phenotypic determination (≤0.25 µg/mL), and 100% (36.8%) were azithromycin susceptible based on the phenotypic determination (≤1 µg/mL) (Supplementary Table 3).

Since multiple loci may contribute to antimicrobial susceptibility and isolates with susceptibility-associated alleles at all molecular markers were rare, we also used regression analysis to generate equations to predict MIC from multiple molecular
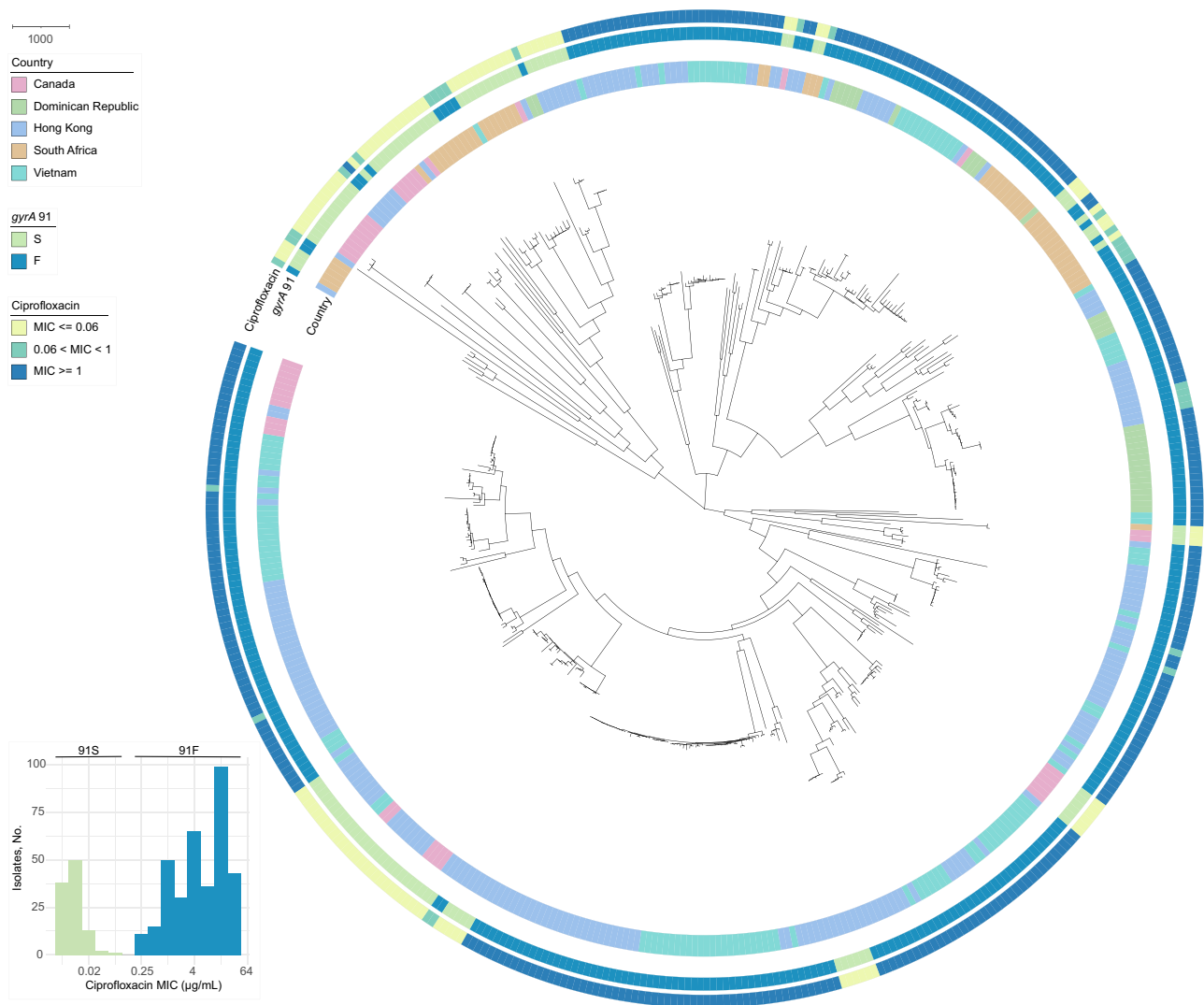
Figure 1. The *gyrA* 91S genotype is associated with ciprofloxacin susceptibility across geographic locations. A maximum likelihood whole-genome phylogeny of 457 *Neisseria gonorrhoeae* isolates mapped to the NCCP11945 (NC_011035.1) reference genome was generated using Gubbins software. Branch lengths represent nonrecombinant substitutions. Inner annotation ring represents the geographic locations of isolates: Canada (*pink*), Dominican Republic (*green*), Hong Kong (*blue*), South Africa (*tan*), or Vietnam (*teal*). Middle ring represents *gyrA* codon 91: serine, S (*green*) or phenylalanine, F (*blue*), and outer ring, the ciprofloxacin minimum inhibitory concentration (MIC) category: ≤0.06 μg/mL (*yellow*), 0.06 to <1 μg/mL (*green*), or ≥1 μg/mL (*blue*). Inset depicts the distribution of ciprofloxacin MICs; isolates with the *gyrA* 91S genotype have ciprofloxacin MICs ranging from 0.008–0.06 μg/mL, and isolates with the *gyrA* 91F genotype have ciprofloxacin MICs ranging from 0.25 − >32 μg/mL.

markers (Supplementary Figure 1). We found some variance in the data such that our predicted MICs did not perfectly match measured MICs (Supplementary Figure 2); however, the PPVs were >90% for each antimicrobial (Supplementary Table 4). In addition, we used the regression equations reported by Demczuk et al [44] to create a second set of predicted MICs that we compared to our measured MICs (Supplementary Figure 3). We found that these equations also performed well with PPVs >95% for ciprofloxacin, ceftriaxone, cefixime and azithromycin susceptibility (Table 3). However, using both sets of equations, we found that some isolates with nonsusceptible MICs were incorrectly predicted to be susceptible for some

antimicrobials. Using the equations from Demczuk et al [44], that was 4.2% of isolates for cefixime, 1.1% for ceftriaxone and 0.5% for azithromycin (Table 2 and Supplementary Table 4).

DISCUSSION

We collected and analyzed *N. gonorrhoeae* isolates from multiple geographic sites around the world to assess the performance of WGS and bioinformatic analysis of known genetic markers to predict antimicrobial susceptibility. Encouragingly, all isolates in our diverse collection were susceptible to zoliflodacin
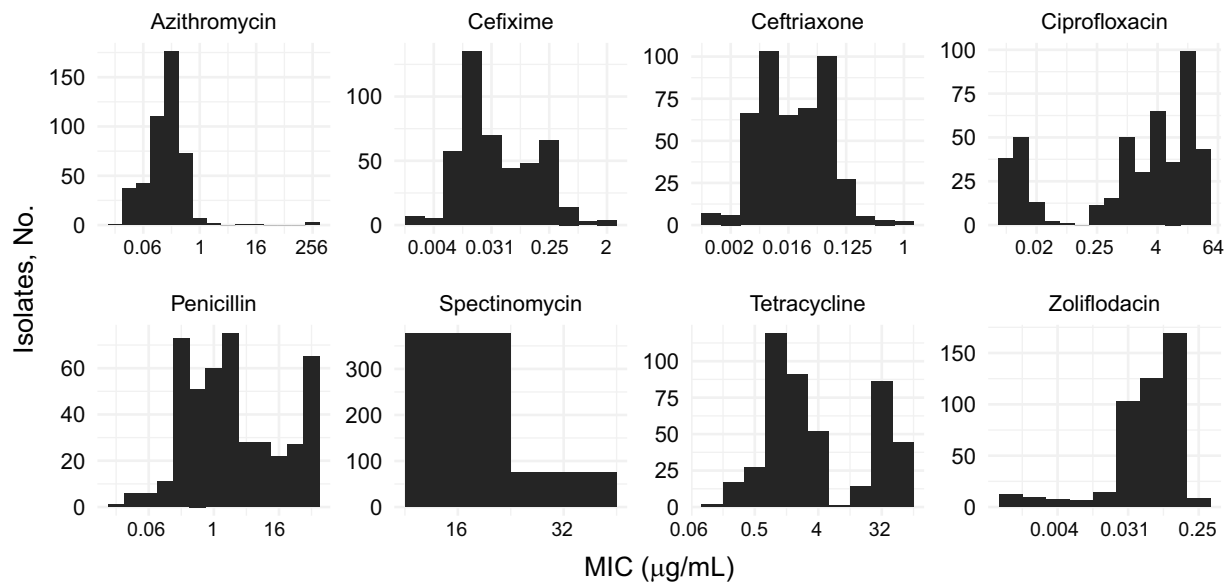
Figure 2. Histograms indicating the azithromycin, cefixime, ceftriaxone, ciprofloxacin, penicillin, spectinomycin, tetracycline, and zoliflodacin minimum inhibitory concentration (MIC) distribution in the collection of *Neisseria gonorrhoeae* isolates (N = 457).

Table 3. Performance of Genotype-Based Prediction of Susceptibility Calculated Using Previously Published Regression EquationsS Compared to the Phenotypic Susceptibility in a Collection of *Neisseria gonorrhoeae* Isolates From 5 Countries (N = 457)[a]

Antimicrobial	MIC Breakpoint, µg/mL	TP	FP	TN	FN	PPV, % Est	LCL[b]
Ciprofloxacin	≤0.06	104	0	353	0	100	97.2
	≤0.5	130	57	268	2	69.5	63.5
Cefixime	≤0.06	255	104	29	67	71.0	66.8
	≤0.125	370	83	1	1	81.7	78.4
	≤0.25	436	19	0	0	95.8	93.9
Ceftriaxone	≤0.06	260	2	33	160	99.2	97.6
	≤0.125	366	5	4	80	98.7	97.2
	≤0.25	449	5	0	1	98.9	97.7
Azithromycin	≤0.5	409	5	9	30	98.8	97.5
	≤1	437	2	5	9	99.5	98.6
Penicillin	≤0.06	0	0	442	13	…	…
	≤1	110	3	241	101	97.3	93.3
Tetracycline	≤0.06	0	0	434	19	…	…
	≤1	19	0	289	145	100	85.4

Abbreviations: FN, false-negative; FP, false-positive; LCL, lower confidence limit for 95% confidence interval; MIC, minimum inhibitory concentration; TN, true-negative; TP, true-positive.

[a]Susceptibility predictions were based on regression equations from Demczuk et al [44] and were compared with phenotypically measured susceptibility.

[b]LCL based on 1-sided 95% Clopper-Pearson confidence interval.

by an MIC breakpoint of ≤0.25 µg/mL, confirming a large prior in silica study [21]. Our results affirm previous findings and indicate that *N. gonorrhoeae* diagnostics based on the *gyrA* genotype will perform well across geographic locations [26, 27]. In all isolates, *gyrA* 91S predicted ciprofloxacin susceptibility

defined by an MIC of ≤0.06 µg/mL. In addition, there was 100% agreement between those with *gyrA* 91F and nonsusceptibility to ciprofloxacin defined by the CLSI breakpoint of MIC >0.06 µg/mL. The PPV for susceptibility is the most important factor when considering using genetic markers for susceptibility determination, because a high PPV indicates that the genetic marker can reliably be used in clinical contexts to indicate susceptibility (eg, wild-type *gyrA* codon 91S in the case of ciprofloxacin) and the expectation of successful treatment [46].

Single marker prediction did not perform as well for any of the other antimicrobials we evaluated. Moreover, few isolates had all of the molecular markers associated with susceptibility, and thus it would be rare that testing for susceptible genotypes at all markers would be a useful way to inform therapy selection for *N. gonorrhoeae* infection. However, equations derived from linear regression performed relatively well in predicting susceptibility to antimicrobials; there were some cases of false-positive susceptible determination for a small percentage of isolates for ceftriaxone, cefixime, and azithromycin (<5% demonstrating susceptibility when the measured MIC is above the CLSI breakpoint for susceptibility). Those isolates for which the regression equation–predicted MIC indicated susceptibility but that were not deemed susceptible by phenotypic characterization may have undescribed genetic variants or more complicated molecular interactions causing contributing to the observed high MICs.

The training data set for genotype-based prediction informs performance on test data sets of isolates obtained from other settings. Using a similar regression-based approach and a training data set of 1280 strains, the majority from Canada, a

genotype-based prediction of resistance phenotypes yielded output best for a test data set representing 1095 Canadian strains and somewhat lower correlation among 431 strains from the United States and the United Kingdom [44]. While our data set aimed to reduce the likelihood of bias from restricted geographic sampling, it was neither geographically nor temporally comprehensive. Ongoing monitoring of resistance patterns and emergence of novel determinants of resistance will be critical if molecular AST is introduced [47].

Ceftriaxone is the recommended empiric treatment for *N. gonorrhoeae* infection in the United States and other parts of the world, and concern about the emergence of resistance underscores the need for strategies to help maintain its clinical utility. In the current study, we provide evidence supporting the use of genotype-based prediction of antibiotic susceptibility to expand treatment options from empiric ceftriaxone to a range of possible tailored therapies. Such an approach could have several benefits. First, reduction of the selective pressures from ceftriaxone use could slow the emergence of resistance [23, 48, 49]. Second, when isolates are known to be susceptible to ≥2 antibiotics, the availability of choice provides treatment flexibility when there are supply chain challenges and in clinical scenarios where the empiric antibiotic option is not ideal (eg, because of allergies or the mode of administration). The linear regression approach used here could also be used more broadly in molecular surveillance programs to identify *N. gonorrhoeae* strains with unexplained antimicrobial resistance. Continued global surveillance and growing understanding of the genetic basis of resistance, even as new antibiotics are introduced into clinical practice, will be critical for maintaining accuracy and the broad utility of genotype-based diagnostics.

Supplementary Data

Supplementary materials are available at *The Journal of 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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