Characterization of a *Neisseria gonorrhoeae* Ciprofloxacin panel for an antimicrobial resistant Isolate Bank

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Abstract

**Objectives**

*Neisseria gonorrhoeae* (gonococcus) infection is one of the most commonly reported nationally notifiable conditions in the United States. Gonococcus has developed antimicrobial resistance to each previously used antibiotic for gonorrhea therapy. However, some isolates may be still susceptible to no longer recommended, yet still effective antibiotics. This in turn suggests that targeted therapy could slow resistance development to currently recommended empirical treatments. We curated a gonococcal Ciprofloxacin Antibiotic Resistance Isolate Bank panel (Cipro-panel) as a tool for validating or developing new tests to determine ciprofloxacin susceptibility.

**Method**

The Cipro-panel was selected using whole genome sequencing, bioinformatic tools, and antimicrobial susceptibility testing (AST) data. Isolates were further selected based on nucleotide variations in *gyrA* and *parC* genes.

**Results**

We selected 14 unique *N. gonorrhoeae* isolates from the 2006–2012 Gonococcal Isolate Surveillance Project (GISP) collection. They represented a wide range of antimicrobial susceptibility to ciprofloxacin and commonly observed nucleotide variations of *gyrA* and *parC* genes. This Cipro-panel consists of 5 isolates with resistant phenotypes (MIC > 1 μg/mL), 8 isolates with susceptible phenotypes (MIC < 0.06 μg/mL), and 1 isolate failing in the Clinical and Laboratory Standards Institute defined intermediate range. Among the *gyrA* variations we observed a total of 18 SNPs. Four positions had nonsynonymous changes (nucleotide positions 272, 284, 1093, and 1783). The first two positions (272 and 284) have been linked previously with resistance to ciprofloxacin (i.e. amino acid positions 91 and 95). For the *parC* gene, we observed a total of 21 possible SNPs. Eight of those SNPs resulted...
in non-synonymous amino acid changes. One location (amino acid 87) has been previously reported to be associated with ciprofloxacin resistance.

Conclusions
This Cipro-Panel is useful for researchers interested in developing clinical tests related to ciprofloxacin. It could also provide additional choices for validation, quality assurance purposes and improve antibiotic usage.

Introduction

*Neisseria gonorrhoeae* (gonococcus) is the causative agent of gonorrhea, one of the nationally notifiable conditions in the United States and one of the most commonly reported Sexually Transmitted Diseases in the world [1, 2]. *Neisseria gonorrhoeae* is an exclusive human pathogen and is well-adapted to the genital system of the human. However, it can cause infections in both male and female reproductive systems, the pharynx, the rectum, and other anatomical sites (e.g. joints) as a disseminated infection. The organism is genetically versatile in its ability to develop drug resistance, thus development of resistance to antimicrobial agents by the gonococcus is a major public health concern. In the past, while retaining previously acquired antimicrobial resistance, *N. gonorrhoeae* developed resistance toward all first-line drugs used in the standard treatment. These drugs include commonly used antibiotics such as penicillin, tetracycline, macrolides, and fluoroquinolones such as ciprofloxacin [3–7]. Because of the gradual increase in proportion of isolates with higher minimum inhibitory concentrations (MIC) toward the current first line class of drug, cephalosporins, in 2012 CDC recommended use of dual-antibiotic therapy consisting of an injectable cephalosporin (ceftriaxone) and one oral dose of a macrolide (azithromycin) as the regimen to treat uncomplicated gonococcal infections [2, 4]. At the end of 2020, Centers for Disease Control and Prevention’s (CDC) new treatment guidelines removed azithromycin from its recommendations partly because the percentage of *N. gonorrhoeae* isolated with reduced susceptibility to azithromycin (MIC ≥2.0 μg/mL) increased more than sevenfold over 5 years (from 0.6% in 2013 to 4.6% in 2018). Ceftriaxone is now recommended as a monotherapy for non-complicated gonorrhea [5].

The fluoroquinolone class antibiotic ciprofloxacin was recommended by CDC’s treatment guideline as the first-line treatment option for gonorrhea from 1996 to 2006. Despite the fact that some states in the United States such as Hawaii and California have discontinued the use of ciprofloxacin for gonorrhea treatment, however, not until 2007 ciprofloxacin was discontinued recommended by CDC as the first option for treating non-complicated gonorrhea [3, 4]. The principle for this decision was based on a recommendation by the WHO that when the microbial resistance rate toward an antibiotic reaches 5% in the population, the drug may be removed from use [8–11]. Fluoroquinolones exert their activity by inhibiting the replication of gonococci through interference of the binding of DNA gyrase and topoisomerase. Drug resistance toward ciprofloxacin thus developed through mutations in DNA gyrase (GyrA) and topoisomerase IV subunits (ParC) [6, 7, 12].

In response to the concerns of increasing antibiotic resistant isolates, CDC published a threat report ranking drug resistant *N. gonorrhoeae* as “Urgent Threat” [9]. An important effort is to develop tools that can enhance the detection of antibiotic resistance in gonorrhea locally, nationally, and internationally. To this end, a *N. gonorrhoeae* Ciprofloxacin Antibiotic Resistance Isolate Bank panel (Cipro-panel) was curated and made available through the CDC.
In this panel, isolates were whole genome sequenced, characterized, and susceptibility to ciprofloxacin was documented. We hope this panel can help developing advanced point of care tests to quickly identify infections that are still susceptible to ciprofloxacin.

**Methods**

**Bacteria strains**

*Neisseria gonorrhoeae* isolates were propagated on GC base medium with 1% IsoVitalex and 5% FBS (SRP, Scientific Resources Program, CDC) at 36±1°C supplemented with 5% CO2 for 20 to 24 hours. A 300–500 ul culture in trypticase soy broth (TSB) containing 20% glycerol (SRP, CDC) was kept at -70°C. Isolates included in this Cipro-panel were characterized using standard microbiological methods. Species identification was confirmed using the AP-NHI strips (Analytical Profile Index for *Neisseria* and *Haemophilus*; bioMerieux, France). The species identification of each isolate was further verified using matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF) following manufacturer’s recommendation (Bruker Microflex Biotyper, Billerica, MA) [14].

**Antimicrobial susceptibility testing (AST)**

The ciprofloxacin agar dilution method was performed according to the Clinical and Laboratory Standards Institute (CLSI) M07 protocol [15, 16] and following the Clinical Laboratory Improvement Amendments (CLIA) regulations. The Etest method was used as additional verification and performed as previously described [17]. The breakpoints and determination of susceptibility (S), intermediate range (I), and resistance (R) were based on CLSI criteria M100 [16]. In brief, the agar dilution and Etest methods were prepared by suspending colonies of *N. gonorrhoeae* from an overnight Chocolate II agar plate (SRP, CDC) into Mueller-Hinton broth (Difco Laboratories, Fisher Scientific, MI) and adjusted to an optical density equal to that of a 0.5 McFarland standard. The cultures were applied to plates of specific antibiotic concentrations (agar dilution) or streaked to a plate and appropriate antibiotic strips (bioMerieux, France) were applied (Etest). The plates were incubated at 36±1°C in 5% CO2 for 20–24 hours. The minimum inhibitory concentrations (MICs) were interpreted by reading growth inhibition (agar dilution) or the intercept of the inhibition zone around the strip (Etest). The agar dilution MIC values were reported and Etest was used for verification purpose.

**Whole-genome sequencing and analyses**

DNA was extracted using the Promega Genomic DNA Purification Kit (Promega, Madison, WI) and whole-genome sequencing was performed using a standard protocol [18, 19]. Specifically, libraries were prepared using the NEB Genome Library Preparation Kit (New England Biolab, MA) and sequenced as paired-end 2x250 bp reads using the Illumina MiSeq platform (Illumina, CA). Preprocessing assessed the read quality with Trim_Galore (v 0.3.7) which contains FastQC and Cutadapt [20] to perform quality assessment, remove duplicate reads and trimming of reads. The quality of the genome was evaluated using QUAST (v 4.3) and assembled using SPAdes (v 3.9.0) [21, 22]. Finally, annotation was completed using NCBI’s Prokaryotic Genome Annotation Pipeline [23, 24]. Reads were mapped to the FA1090 reference sequence (GenBank accession number NC002946). SAMtools was used to convert the alignments and using GATK IndelRealigner command. Pilon was further used to call the variants and raw variants were filtered by using snpSift with depth > = 20 and genotype quality score > = 200. Additionally, *gyrA* and *parC* sequences were aligned and compared using the CLC
The assembly metrics and quality control data for WGS results are shown in Table 1.

### Results

#### Selection criteria

Two hundred fifty newly sequenced *N. gonorrhoeae* isolates from the United States’ Gonococcal Isolate Surveillance Project (GISP; [25, 26]) collected from year 1999 to 2012 and archived at the CDC were used to select this panel. Based on the unique *gyrA* and *parC* sequence variations together with their ciprofloxacin susceptibility profile, 14 unique isolates were selected and included in this Ciprofloxacin Antibiotic Resistance Isolate Bank panel (Cipro-panel). The isolates included in this Cipro-panel are numbered from 1 to 14 with corresponding AR Bank numbers 963–976. Their MIC values and the accession numbers of the whole genome sequencing results are available online (Table 1) [13].

### Genotype characterizations

We observed two well-described mutations in *gyrA* at positions 272 and 284, which caused non-synonymous changes corresponding to amino acid 91 and 95, respectively. We also observed additional mutations at 12 nucleotide positions (Table 2). They are at nucleotide positions, 276, 279, 666, 744, 882, 927, 1032, 1094, 1110, 1722, 1783, and 2433. Among these, positions 1094 and 1783 resulted in amino acid changes. They are arginine to histidine at position 365 (R365H) of panel number 8, and alanine to threonine at position 595 (A595T) of panel number 5. There were no known functional changes associated with these additional amino acid mutations.

The *parC* gene has mutations in 19 nucleotide locations (Table 3) which include the commonly recognized AGT to CGT at position 259 (S87R). Several non-synonymous amino acid changes resulting from nucleotide changes other than position 259 were also recognized. They are at positions 1150 (I384V), 1304/5 (V436A), 1375 (G459S), 1435 (L479F), 1789(V596I), and
There were no known functional changes associated with these additional amino acid mutations.

Phenotypic characterizations and antibiotic susceptibility

Traditionally the GyrA amino acid positions S91 and D95 are considered wild type and representing the ciprofloxacin susceptible phenotypes. Thus, based on CLSI AST ciprofloxacin testing criteria (M100; 17), 8 isolates have the susceptible phenotype (MIC $\leq 0.06$ μg/mL; wild-type S91/D95: panel numbers 3, 4, 5, 6, 7, 8, 10, 11); 5 isolates have a resistant phenotype (MIC $> = 1$ μg/mL; panel numbers 2, 12, 13, 14, F91/G95; No 9, F91/A95), and one isolate has the intermediate phenotype (0.12 μg/mL $< =$ MIC $< =$ 0.5 μg/mL; panel number 1, F91/G95).

For the ParC amino acid composition, ten isolates have the ParC wild-type (S87, Table 3) and four have the mutant phenotype of R87 (No. 2, 12, 13, 14). Combined, eight isolates are wildtype for both GyrA and ParC (Nos 3, 4, 5, 6, 7, 8, 10, 11). Six isolates have a combined amino acid variation at either GyrA or ParC. Among these, number 1 has a F91/G95/S87 (here referred to as FGS) combination; numbers 2, 12, 13, and 14 have an FGR combination, and number 9 has the FAS combination.

Supplemental genetic profiles of the Cipro-panel

A more detailed genetic mutational analyses is included in Table 4a–4f) as a supplement to this collection. This table was created using CDC’s Drug-Resistant Gonorrhea Genome Profiler version 2.9.2 [CDC, accessible online: https://amdportal-sams.cdc.gov/].

Discussion

Antimicrobial drug resistance is a global emergency. The rapid development of antibiotic resistance in *N. gonorrhoeae* has the potential to reduce the clinical utility of nearly any antibiotic used for treatment within a few years of its introduction. Since 2007, CDC has discontinued recommending ciprofloxacin as anti-gonorrhea treatment 8 years since its initial
| Bank No | MIC (μg/ml) | gyrA aa91 | gyrA aa95 | parC aa87 | gyrase A mutation | Location of Nucleotide Mutation |
|---------|-------------|-----------|-----------|-----------|----------------|-------------------------------|
| 1       | 0.5         | G         | G         | S         | AGT            | ATG                           |
| 2       | 32          | F         | G         | R         | TAT            | GTC                           |
| 3       | 0.004       | S         | D         | S         | AGT            | GTG                           |
| 4       | 0.008       | S         | D         | S         | AGT            | GTG                           |
| 5       | 0.008       | S         | D         | S         | AGT            | GTG                           |
| 6       | 0.004       | S         | D         | S         | AGT            | GTG                           |
| 7       | 0.004       | S         | D         | S         | AGT            | GTG                           |
| 8       | 0.004       | S         | D         | S         | AGT            | GTG                           |
| 9       | 8           | F         | A         | S         | AGT            | GTG                           |
| 10      | 0.008       | S         | D         | S         | AGT            | GTG                           |
| 11      | 32          | F         | G         | R         | TAT            | GTC                           |
| 12      | 32          | F         | G         | R         | TAT            | GTC                           |
| 13      | 32          | F         | G         | R         | TAT            | GTC                           |
| 14      | 32          | F         | G         | R         | TAT            | GTC                           |

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Table 4. Complete genetic profile based on Gonorrhea Genome Profiler v2.9.2.

**A**

| Cip Panel ID | AR Bank ID | CIP MIC | PEN MIC | TET MIC | CRO MIC | CFM MIC | AZN MIC | GEN MIC | Beta-lactamase | 23S-2611 base | 23S-2059 base | 23S-2058 base | 23S-2058 freq | mtrR mosaic | mtrR promoter |
|--------------|------------|---------|---------|---------|---------|---------|---------|---------|----------------|----------------|----------------|----------------|---------------|---------------|---------------|
| 1            | 963        | 0.38    | 1       | 1       | 0.004   | 0.008   | 0.25    | 4       | Positive       | A              | A              | A              | 1             | FALSE         | DEL            |
| 2            | 964        | >32     | 2       | 2       | 0.015   | 0.03    | 25      | 4       | Negative       | A              | A              | A              | 1             | FALSE         | DEL            |
| 3            | 965        | 0.04    | 1       | 1       | 0.004   | 0.015   | 0.25    | 4       | Negative       | T              | A              | A              | 1             | FALSE         | A              |
| 4            | 966        | 0.08    | 0.25    | 1       | 0.004   | 0.008   | ≥16     | 4       | Negative       | C              | G              | A              | 1             | FALSE         | A              |
| 5            | 967        | 0.004   | 0.06    | 0.06    | ≤0.001  | 0.004   | 0.125   | 4       | Negative       | C              | A              | A              | 1             | FALSE         | A              |
| 6            | 968        | 0.004   | 0.25    | 0.5     | 0.002   | 0.008   | 0.125   | 4       | Negative       | C              | A              | A              | 1             | FALSE         | A              |
| 7            | 969        | 0.004   | 0.25    | 0.25    | 0.004   | 0.008   | 0.125   | 4       | Negative       | C              | A              | A              | 1             | FALSE         | A              |
| 8            | 970        | 0.004   | 0.5     | 0.5     | 0.008   | 0.03    | 0.06    | 4       | Negative       | C              | A              | 1              | 1             | FALSE         | A              |
| 9            | 971        | 6       | 6       | 4       | 0.015   | 0.03    | 25      | 2       | Negative       | C              | A              | 1              | 1             | FALSE         | DEL            |
| 10           | 972        | 0.004   | 0.25    | 1       | 0.004   | 0.015   | 2       | 4       | Negative       | C              | A              | 1              | 1             | FALSE         | C              |
| 11           | 973        | 0.002   | 0.5     | 16      | 0.008   | 0.03    | 0.06    | 4       | Negative       | C              | A              | 1              | 1             | FALSE         | A              |
| 12           | 974        | >32     | 2       | 2       | 0.03    | 0.125   | 2       | 4       | Negative       | C              | A              | 1              | 1             | FALSE         | DEL            |
| 13           | 975        | 16      | 2       | 2       | 0.015   | 0.03    | 0.25    | 4       | Negative       | C              | A              | 1              | 1             | FALSE         | DEL            |
| 14           | 976        | 32      | 2       | 2       | 0.015   | 0.03    | 0.25    | 4       | Negative       | C              | A              | 1              | 1             | FALSE         | DEL            |

**B**

| Cip Panel ID | AR Bank ID | mtr120 promoter | mtrR aa39 | mtrR aa44 | mtrR aa45 | mtrR aa47 | mtrR aa79 | mtrR aa105 | mtrR premature stop | penA allele | penA aa311 | penA aa312 | penA aa316 | penA aa483 | penA aa501 |
|--------------|------------|-----------------|-----------|-----------|-----------|-----------|-----------|------------|---------------------|-------------|------------|------------|------------|-------------|------------|
| 1            | 963        | G               | G         | A         | R         | D         | L         | D         | H                   | FALSE       | 2.001      | A          | V          | T          | A          |
| 2            | 964        | G               | G         | A         | R         | G         | L         | D         | Y                   | FALSE       | 12.001     | A          | I          | V          | T          |
| 3            | 965        | G               | G         | A         | R         | D         | L         | D         | H                   | FALSE       | 2.001      | A          | I          | V          | T          |
| 4            | 966        | G               | G         | A         | R         | D         | L         | D         | H                   | FALSE       | 2.001      | A          | I          | V          | T          |
| 5            | 967        | G               | G         | A         | R         | G         | L         | N         | Y                   | FALSE       | 15.001     | A          | I          | V          | T          |
| 6            | 968        | G               | G         | A         | R         | G         | L         | D         | H                   | TRUE        | 22.001     | A          | I          | V          | T          |
| 7            | 969        | G               | G         | A         | R         | G         | L         | N         | Y                   | FALSE       | 2.002      | A          | I          | V          | T          |
| 8            | 970        | G               | G         | A         | R         | D         | L         | D         | H                   | FALSE       | 9.001      | A          | I          | V          | T          |
| 9            | 971        | G               | G         | A         | R         | G         | L         | D         | Y                   | FALSE       | 43.001     | A          | I          | V          | T          |
| 10           | 972        | G               | G         | A         | R         | G         | L         | N         | H                   | FALSE       | 2.001      | A          | I          | V          | T          |
| 11           | 973        | G               | G         | A         | R         | G         | L         | D         | H                   | FALSE       | 19.001     | A          | I          | V          | T          |
| 12           | 974        | G               | G         | A         | R         | G         | L         | D         | Y                   | FALSE       | 42.001     | A          | M          | T          | T          |
| 13           | 975        | G               | G         | A         | R         | G         | L         | D         | Y                   | FALSE       | 12.001     | A          | I          | V          | T          |
| 14           | 976        | G               | G         | A         | R         | G         | L         | D         | Y                   | FALSE       | 12.001     | A          | I          | V          | T          |

**C**

| Cip Panel ID | AR Bank ID | penA aa512 | penA aa542 | penA aa545 | penA aa549 | penA aa551 | penA D345ins | penA aa375 | penA aa421 | pilQ full length | pilQ aa341 | pilQ aa526 | pilQ aa648 | pilQ aa666 |
|--------------|------------|------------|------------|------------|------------|------------|--------------|------------|------------|-----------------|------------|------------|------------|------------|
| 1            | 963        | N          | G          | G          | A          | P          | TRUE         | A          | P          | TRUE            | N          | D          | N          | E          |
| 2            | 964        | N          | G          | G          | A          | S          | TRUE         | A          | P          | TRUE            | N          | D          | N          | E          |
| Cip Panel ID | AR Bank ID | gyrA aa91 | gyrA aa95 | parC aa86 | parC aa87 | parC aa88 | parC aa91 | blaTEM present | TeTM present | porB allele | rpsJ aa57 | ftsX aa31 | rplD aa68 | rplD aa70 |
|--------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|---------------|--------------|-------------|----------|----------|---------|---------|
| 1            | 963        | F         | A         | G         | D         | S         | S         | E             | TRUE         | FALSE       | 8        | M        | T        | G        |
| 2            | 964        | F         | A         | G         | D         | R         | S         | E             | FALSE        | FALSE       | 8        | M        | T        | G        |
| 3            | 965        | S         | A         | D         | D         | S         | S         | E             | FALSE        | FALSE       | 3        | M        | T        | G        |
| 4            | 966        | S         | A         | D         | D         | S         | S         | E             | FALSE        | FALSE       | 3        | M        | T        | G        |
| 5            | 967        | S         | A         | D         | D         | S         | S         | E             | FALSE        | FALSE       | 3        | V        | T        | G        |
| 6            | 968        | S         | A         | D         | D         | S         | S         | E             | FALSE        | FALSE       | 3        | M        | T        | G        |
| 7            | 969        | S         | A         | D         | D         | S         | S         | E             | FALSE        | TRUE        | 1        | V        | T        | G        |
| 8            | 970        | S         | A         | D         | D         | S         | S         | E             | FALSE        | FALSE       | 100       | M        | T        | G        |
| 9            | 971        | F         | A         | A         | D         | S         | S         | E             | FALSE        | TRUE        | 4        | M        | T        | G        |
| 10           | 972        | S         | A         | D         | D         | S         | S         | E             | FALSE        | TRUE        | 1        | M        | T        | G        |
| 11           | 973        | S         | A         | D         | D         | S         | S         | E             | FALSE        | TRUE        | 14       | M        | T        | G        |
| 12           | 974        | F         | A         | A         | D         | R         | S         | E             | FALSE        | FALSE       | 11       | M        | T        | G        |
| 13           | 975        | F         | A         | G         | D         | R         | S         | E             | FALSE        | FALSE       | 8        | M        | T        | G        |
| 14           | 976        | F         | A         | G         | D         | R         | S         | E             | FALSE        | FALSE       | 23       | M        | T        | G        |

| Cip Panel ID | AR Bank ID | rplV ins | macA aa99 | mtrD aa42 | mtrD aa46 | mtrD aa48 | mtrD aa101 | mtrD aa174 | mtrD aa612 | mtrD aa662 | mtrD aa714 | mtrD aa821 | mtrD aa823 | macA promoter | norM promoter |
|--------------|------------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----------------|---------------|
| 1            | 963        | FALSE    | D         | T         | H         | I         | N         | R         | F         | V         | R         | S         | K         | A         | C             | G             |
| 2            | 964        | FALSE    | D         | T         | H         | I         | N         | R         | F         | V         | R         | S         | K         | A         | C             | G             |
| 3            | 965        | FALSE    | D         | T         | H         | I         | N         | R         | F         | V         | R         | S         | K         | A         | C             | G             |
| 4            | 966        | FALSE    | D         | N         | R         | T         | D         | R         | F         | I         | R         | A         | E         | G         | C             | G             |
| 5            | 967        | FALSE    | N         | T         | H         | I         | N         | R         | F         | V         | R         | S         | K         | A         | C             | G             |
| 6            | 968        | FALSE    | N         | T         | H         | I         | N         | R         | F         | V         | R         | S         | K         | A         | C             | G             |
| 7            | 969        | FALSE    | D         | T         | H         | I         | N         | R         | F         | V         | R         | S         | K         | A         | C             | G             |
| 8            | 970        | FALSE    | D         | T         | H         | I         | N         | R         | F         | V         | R         | S         | K         | A         | C             | G             |
| Panel ID | AR Bank ID | ermB present | ermC present | ermF present | mefA present | gyrB aa429 | gyrB aa450 | acnB aa348 | acnB aa371 | 16S-1053 base | 16S-1053 freq | 16S-1186 base | 16S-1186 freq | rpsE aa24 | MLST | NG-MAST |
|----------|------------|--------------|--------------|--------------|--------------|------------|------------|------------|------------|---------------|--------------|---------------|--------------|------------|----------|---------|
| 1        | 963        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 2             | 1            | T         | 7367    | 6842    |
| 2        | 964        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 1901    | 225     |
| 3        | 965        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 1580    | 8097    |
| 4        | 966        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 1580    | 649     |
| 5        | 967        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 6962    | 1063    |
| 6        | 968        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 8149    | 1319    |
| 7        | 969        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 0       | -       |
| 8        | 970        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 1893    | -       |
| 9        | 971        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 1600    | 2194    |
| 10       | 972        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 7367    | 1028    |
| 11       | 973        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 8152    | -       |
| 12       | 974        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 1901    | 1407    |
| 13       | 975        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 1901    | 735     |
| 14       | 976        | FALSE        | FALSE        | FALSE        | FALSE        | D          | K          | G          | Q          | 1             | 1            | 1             | 1            | T         | 1901    | 323     |
recommendation [4]. With the advancement of newer, faster rapid molecular tests, there is a potential for clinicians to choose antibiotics discontinued for gonorrhea treatment based on the presence or absence of known genetic markers. This kind of targeted treatment potentially has the advantages of allowing physicians to select common and still effective antibiotics based on testing results [27–29].

Ciprofloxacin, a fluoroquinolone, is widely used to treat many bacterial infections such as pneumonia, meningitis, diarrhea, and urinary tract infections [30, 31]. This is the only class of antibiotic that directly inhibits bacterial DNA synthesis for gonorrhea treatment. It was the main choice for treating gonorrhea from 1998 to 2006 until resistance exceeded 5% and was removed from recommendations [2–4]. The main mutations conferring fluoroquinolone resistance are on the subunits of the DNA gyrase A, GyrA, and topoisomerase IV, ParC [33]. At the molecular level mutations conferring resistance are mainly at nucleotides C272 (amino acid S91) and A284 (amino acid D95) positions of gyrA and at the A259 (amino acid S87) location of the parC [28, 32–34].

Although ciprofloxacin has not been recommended for treating gonorrhea for over 10 years, the reported rates of ciprofloxacin resistant gonococcal isolates remain elevated. In the past ten years (2009–2017), the proportion of resistant isolates among sexually transmitted diseases (STD) clinics has increased steadily but remains below 40% [3, 9, 12, 25]. This also means that about 60% of uncomplicated gonococcal infections in the US are possibly still sensitive to ciprofloxacin. Therefore, ciprofloxacin may be considered for use in patients with confirmed, susceptible gonorrhea when recommended first-line therapy is not tolerated [33, 35]. This notion is especially facilitated by the fact that the ciprofloxacin resistance mechanisms utilized by N. gonorrhoeae are well defined and there are clear targets to be selected for developing rapid molecular tests [28, 29]. To this end, to conserve the usage of current first line antibiotics and in considering re-use of previously favored first line antibiotics, ciprofloxacin is an ideal candidate.

Here we created a panel of 14 gonococcal isolates which has the utility to serve multiple purposes for the scientific community. The mutations described in this panel provide a tool for scientists to develop molecular tests or validate existing tests. For instance, this panel could serve as an internal quality control for interlaboratory studies or external quality assurance for international collaborations. Finally, the isolates in this panel represent the susceptible, intermediate, and resistant phenotypes with respect to established gonococcal ciprofloxacin AST breakpoints.

In conclusion, this ciprofloxacin isolate panel has been extensively characterized based on its ciprofloxacin AST profile and the gyrA and parC genes sequences. This panel should be useful for developing ciprofloxacin susceptibility related tests or for quality assurance purposes [27, 28]. Availability of such tests for detecting ciprofloxacin susceptibility can provide clinicians with tools to improve antibiotic stewardship, potentially reducing costs, and save first line drugs for individuals that truly need it.

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