Gonorrhea, a sexually transmissible infection (STI) caused by Neisseria gonorrhoeae, is the second most common bacterial STI (1). Most gonorrhea cases are mild, but serious complications can occur. Gonorrhea is treated with antibiotics, and the recommended treatment is dual extended-spectrum cephalosporin (ESC)/azithromycin therapy or ceftriaxone monotherapy (2).

One of the main characteristics of N. gonorrhoeae is the plasticity of its genome, favoring the acquisition and dispersion of antimicrobial resistance (AMR). AMR is an increasing issue for gonorrhea treatment, and untreatable gonorrhea represents an imminent global health threat (3).

Whole-genome sequencing (WGS) provides high-resolution data that can support AMR surveillance.

We investigated genomic determinants of antimicrobial resistance in 1,318 Neisseria gonorrhoeae strains isolated in Austria during 2016–2020. Sequence type (ST) 9363 and ST11422 isolates had high rates of azithromycin resistance, and ST7363 isolates correlated with cephalosporin resistance. These results underline the benefit of genomic surveillance for antimicrobial resistance monitoring.

We combined phenotypic AMR testing with WGS to investigate 1,318 N. gonorrhoeae strains isolated in Austria during 2016–2020 and identify genetic risk factors associated with AMR.

The Study
This study encompassed 1,318 N. gonorrhoeae isolates collected in Austria during 2016–2020; isolates were available at the National Reference Centre for Gonococci. We tested all isolates for phenotypic resistance to azithromycin, cefixime, ceftriaxone, ciprofloxacin, tetracycline, and benzylpenicillin, as well as production of β-lactamase (i.e., cefinase positive) (Appendix, https://wwwnc.cdc.gov/EID/article/28/8/22-0071-App1.pdf). We followed European Committee on Antimicrobial Susceptibility Testing guidelines (4) to determine MIC thresholds used in this study.

We performed genomic DNA isolation, WGS, assembly, and contig filtering as described previously (5) (Appendix). We deposited raw reads in the National Center for Biotechnology Information Sequence Read Archive (project no. PRJNA771206). We obtained sequences types (STs) from WGS data by using the PubMLST schemes (6,7). We generated a local N. gonorrhoeae core-genome multilocus sequence typing (cgMLST) scheme with SeqSphere+ target definer tool version 6.0.0 (Ridom, ttps://www.ridom.de) (7) (Appendix). We investigated AMR genes by using allele libraries based on PathogenWatch in TOML format version 0.0.14 (8).

We performed time series analysis, linear regression, univariate analysis, multivariate analysis (logistic regression), and data visualization by using R version 4.0.4 (Appendix). We defined statistical significance as p<0.05. We computed neighbor-joining associations of phylogenomic relatedness among Neisseria gonorrhoeae strains with antimicrobial resistance, Austria, 2016–2020

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DOI: https://doi.org/10.3201/eid2808.220071
trees in SeqSphere+ by using the number of cgMLST allelic differences and exported the trees into R.

We classified isolates according to AMR (Figure 1, panel A; Table) and determined MIC distributions (Figure 1, panel B). We observed high levels of resistance to ciprofloxacin (60%) and tetracycline (46%) (Figure 1, panel A), which increased 5% per year for ciprofloxacin ($p<0.0001$) and 6% per year for tetracycline ($p<0.0001$). The percentage of penicillin-resistant isolates was 16% and decreased over the study period (2% per year; $p<0.0001$) (Figure 1, panel C); 14% of isolates were cefinase-positive, which increased by 2.7% per year ($p<0.0001$).

We detected azithromycin resistance in 9% of the isolates, which increased by 5% per year ($p<0.0001$) (Figure 1). Two isolates from 2020 exhibited high levels of azithromycin resistance (MIC $>256$ µg/mL) but no other AMR. Resistance to ESC was rare; only 3% of isolates were resistant to cefixime, none were resistant to ceftriaxone, and 2.5% had reduced susceptibility to ceftriaxone (MIC $>0.032$ µg/mL). Cefixime resistance decreased by 0.9% per year ($p<0.0001$). Among cefixime-resistant isolates, 23/35 were resistant to ciprofloxacin and penicillin, qualifying as multidrug resistant.

The isolates belonged to 119 different STs in multilocus sequence typing, including 23 newly defined (STs 15803–15825). The most prevalent STs were ST7363 (170 isolates), ST9363 (151 isolates), and ST8156 (113 isolates), which comprised 33% of the isolates. We identified 215 NG-MAST types for 873/1,318 isolates; the most prevalent STs were 12302 (73 isolates), 5441 (59 isolates), and 387 (50 isolates).

Figure 1. Antimicrobial resistance in 1,318 Neisseria gonorrhoeae isolates, Austria, 2016–2020. A) Number of isolates classified as susceptible, intermediate, or resistant. For ceftriaxone, isolates with reduced susceptibility are indicated in blue. For cefixime, β-lactamase producing isolates are indicated as positive (yellow). B) Boxplots of MIC obtained by Etest. Dashed lines indicate the thresholds used to classify the isolates as susceptible, intermediate, or resistant for ciprofloxacin, tetracycline, and penicillin, as susceptible or resistant for azithromycin, cefixime, and as susceptible, reduced susceptibility, or resistant for ceftriaxone. Horizontal lines within boxes indicate median, box tops and bottoms indicate quartiles 1 and 3, and dots indicate potential outliers. C) Evolution of the frequency of resistant isolates over time. Plain lines indicate the 13-week moving average of the percentage of isolates classified as resistant. Trends over time (obtained by linear regression) are represented by the dashed lines.
cgMLST showed a branch including isolates with no or little AMR (Figure 2). We found no clear correlation with the cgMLST classification for penicillin, cefinase, tetracycline, and ciprofloxacin resistance. All cefixime-resistant isolates belonged to a single branch of ST7363 isolates, which also contained 24/32 isolates with reduced susceptibility to ceftriaxone. This branch had above average rates of ciprofloxacin, tetracycline, and penicillin resistance. A branch containing ST9363 and ST11422 isolates had a high rate of azithromycin resistance.

We searched isolate sequences for genes and point mutations associated with AMR (Appendix Table 3). For ciprofloxacin resistance, gyrA D95 substitutions were the main risk factor (adjusted odds ratio [aOR] 7.56 [95% CI 2.33–33.1]) and explained >99% of ciprofloxacin resistance. Tetracycline resistance was strongly associated with tetM carriage (aOR 157 [95% CI 48–965]), which we found in 33% of tetracycline-resistant isolates. For β-lactams, the main risk factor was blaTEM carriage (aOR 67.9 [95% CI 35.2–139]) for penicillin and aOR 234 [95% CI 93.3–683] for cefinase. Mutations in penA were also associated with cefinase positivity (aOR 35.6 [95% CI 14–97.4]).

We found mutations in the macAB promoter or mosaic mtr genes in 138/149 azithromycin-resistant isolates (93%). All cefixime-resistant isolates carried penA G545S substitution. The major risk factor for reduced susceptibility to ceftriaxone was penA A501T/V (aOR 73.9 [95% CI 6.9–3,170]).

Table. Antimicrobial resistance classification and mean MIC of 1,318 Neisseria gonorrhoeae isolates, Austria, 2016–2020

| Antibiotic | Antimicrobial resistance | No. isolates | Total no. isolates* | Frequency, % |
|------------|--------------------------|--------------|---------------------|--------------|
| Azithromycin | Susceptible (<1)        | 1,180        | 1,302               | 90.6         |
|             | Resistant (>1)          | 122          | 1,302               | 9.4          |
|             | MIC, µg/mL              |              | 0.8432 (0.2937–1.3927) |             |
| Cefixime    | Susceptible (<0.125)    | 1,276        | 1,311               | 97.3         |
|             | Resistant (>0.125)      | 35           | 1,311               | 2.7          |
|             | MIC, µg/mL              |              | 0.0289 (0.0266–0.0311) |             |
| Ceftriaxone | Susceptible (<0.032)    | 1,279        | 1,312               | 97.5         |
| Reduced Sensitivity (>0.032) | | 33          | 1,312               | 2.5          |
|             | Resistant (>0.125)      | 0            | 1,312               |              |
|             | MIC, µg/mL              |              | 0.007 (0.0064–0.0076) |             |
| Ciprofloxacin | Susceptible (<0.032)   | 528          | 1,311               | 40.3         |
| Intermediate |                         | 1            | 1,311               | 0.1          |
|             | Resistant (>0.064)      | 782          | 1,311               | 59.6         |
|             | MIC, µg/mL              |              | 6.4455 (5.8446–7.0463) |             |
| Tetracycline | Susceptible (<0.5)     | 431          | 1,208               | 35.7         |
| Intermediate |                         | 215          | 1,208               | 17.8         |
|             | Resistant (>1)          | 562          | 1,208               | 46.5         |
|             | MIC, µg/mL              |              | 7.0349 (5.9602–8.1096) |             |
| Penicillin  | Susceptible (<0.064)    | 246          | 1,312               | 18.8         |
| Intermediate |                         | 861          | 1,312               | 65.6         |
|             | Resistant (>1)          | 205          | 1,312               | 15.6         |
|             | MIC, µg/mL              |              | 2.2397 (1.8598–2.6196) |             |
| Cefinase    | Negative                | 1,083        | 1,266               | 85.5         |
| Positive    |                         | 183          | 1,266               | 14.5         |
| All         |                         | 1,318        | 100                 |              |

*Total number of isolates for which variable data were available.

Conclusions

This study combined phenotypic AMR and genomic data to analyze N. gonorrhoeae strains isolated in Austria during 2016–2020. We used a convenience sample (National Reference Centre for Gonococci collection) and results should be interpreted in light of this limitation. The percentage of N. gonorrhoeae strains resistant to azithromycin, ciprofloxacin, and tetracycline, or producing β-lactamase was increasing during the study period. The rate of azithromycin resistance rate was >13% during 2019–2020, which was high considering that an azithromycin/cefixime combination is a standard treatment for gonorrhea (2). We found no ceftriaxone-resistant isolates, and cefixime resistance rate was low.

We performed isolate typing by using multilocus sequence typing, N. gonorrhoeae multiantigen sequence typing (NG-MAST), and cgMLST. Only 37 isolates belonged to ST1901, which was predominant in isolates from Austria in a European study in 2013, highlighting the fast diversification of N. gonorrhoeae (9). The most common NG-MAST type was 12302; all isolates belonged to ST9363 and 71% were resistant to azithromycin. NG-MAST type 12302 and ST9363 have been associated with azithromycin resistance in other studies (10,11). cgMLST classification highlighted 3 branches with specific AMR patterns: 1 with low rates of AMR, 1 including azithromycin-resistant isolates, and 1 including ESC-resistant isolates. Previous studies comparing AMR and phylogenomic distributions in
different countries showed either that azithromycin/ESC resistance emerged repeatedly in different networks or that their spread was largely clonal (12,13). In Austria, azithromycin and ESC resistance clustering was in favor of single introductions. The use of cgMLST among available classification methods has limitations (i.e., no counting of mutations within 1 gene, exclusion of intergenic regions, and resolution) but also advantages (i.e., no correction of recombination events necessary and one scheme fitting all isolates). This tool corresponds to the need for surveillance, where its lower resolution does not have a major effect.

Figure 2. Correlation between population structure and antimicrobial resistance in Neisseria gonorrhoeae isolates, Austria, 2016–2020. Dendrogram was computed from the distance matrix of the core-genome multilocus sequence typing analysis (N = 1,304). Rims indicate the isolate classification as susceptible, intermediate, or resistant. For ceftriaxone, isolates with reduced susceptibility are indicated in blue. For cefdinir, β-lactamase producing isolates are indicated as positive (yellow). The outer rim indicates sequence types corresponding to ≥2 consecutive isolates. Three branches with specific antimicrobial resistance patterns are indicated. AMR, antimicrobial resistance; AZI, azithromycin; ESC, extended-spectrum cephalosporin.
We used our WGS data to search for genetic determinants of AMR (8,14). Ciprofloxacin resistance matched well with gyrA mutations (9,12). Tetracycline resistance correlated with tetM, and penicillin resistance correlated blaTEM Mutations in penA and mtrR were associated with ESC resistance. Neither substitution C1192U in 16S rDNA nor rpsE V25 mutations, associated with spectinomycin resistance, were found, suggesting a low prevalence of spectinomycin resistance.

Our study provides an overview of the N. gonorrhoeae strains circulating in Austria and their evolution over the past 5 years, both at the phenotypic and genomic level. It also underlines the benefits of genomic surveillance of N. gonorrhoeae, which can support epidemiologic investigations and provide information on specific genes and alleles thought to confer AMR (14).

Acknowledgments
We thank the staff of the Gonococi National Reference Centre and other partner institutions for the collection, isolation, and characterization of the study isolates. We thank Loredana Ingrosso for her feedback throughout the project and on the manuscript.

All co-authors in this manuscript declare that no funding was received from any funding agency in the public, commercial, or not-for-profit sectors. J.S. was supported by a grant from the European Public Health Microbiology Training Programme, European Centre for Disease Prevention and Control (grant agreement no. 1 ECD.7550, implementing ECDC/GRANT/2017/003).

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References
1. Rowe Jr, J. Vander Hoon S, Korenromp E, Low N, Unemo M, Abu-Raddad LJ, et al. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull World Health Organ. 2019;97:548–562P. https://doi.org/10.2471/BLT.18.228486
2. World Health Organization. WHO guidelines for the treatment of Neisseria gonorrhoeae. Report no. 978–92–4–154969–1. 2016 [cited 2022 Jan 8]. https://apps.who.int/iris/bitstream/handle/10665/246114/9789241549691-eng.pdf
3. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. 2017 [cited 2022 Jan 8]. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed
4. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 11.0. 2021 [cited 2022 Jan 8]. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables_v.10.0_Breakpoint_Tables.pdf
5. Hirk S, Lepuschitz S, Cabal Rosel A, Huhulescu S, Blaschitz M, Stöger A, et al. Draft genome sequences of interpatient and intrapatient epidemiologically linked Neisseria gonorrhoeae isolates. Genome Announc. 2018;6:e00319–18. https://doi.org/10.1128(genomeA.00319-18
6. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci U S A. 1998;95:3140–5. https://doi.org/10.1073/pnas.95.6.3140
7. Martin IM, Ison CA, Aanensen DM, Fenton KA, Spratt BG. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J Infect Dis. 2004;189:1497–505. https://doi.org/10.1086/383047
8. Sánchez-Busó L, Yeats CA, Taylor B, Goater RJ, Underwood A, Abudahab K, et al. A community-driven resource for genomic epidemiology and antimicrobial resistance prediction of Neisseria gonorrhoeae at Pathogenwatch. Genome Med. 2021;13:61. https://doi.org/10.1186/s13073-021-00858-2
9. Harris SR, Cole MJ, Spiteri G, Sánchez-Busó L, Golparian D, Jacobsson S, et al.; Euro-GASP study group. Public health surveillance of multidrug-resistant clones of Neisseria gonorrhoeae in Europe: a genomic survey. Lancet Infect Dis. 2018;18:758–68. https://doi.org/10.1016/S1473-3099(18)30225-1
10. Sawatzky P, Demczuk W, Lefevbre B, Allen V, Diggle M, Hoang L, et al. Increasing azithromycin resistance in Neisseria gonorrhoeae due to NG-MAST 12302 clonal spread in Canada, 2015 to 2018. Antimicrob Agents Chemother. 2022;66:e0168821. https://doi.org/10.1128/aac.01688-21
11. Williamson DA, Chow EPF, Gorrie CL, Seemann T, Ingle DJ, Higgins N, et al. Bridging of Neisseria gonorrhoeae lineages across sexual networks in the HIV pre-exposure prophylaxis era. Nat Commun. 2019;10:3988. https://doi.org/10.1038/s41467-019-12053-4
12. Lee RS, Seemann T, Heffernan H, Kwong JC, Gonçalves da Silva A, Carter GP, et al. Genomic epidemiology and antimicrobial resistance of Neisseria gonorrhoeae in New Zealand. J Antimicrob Chemother. 2018;73:353–64. https://doi.org/10.1093/jac/dkx405
13. Harrison OB, Cehovin A, Skett J, Jolley KA, Massari P, Genco CA, et al. Neisseria gonorrhoeae population genomics: use of the gonococcal core genome to improve surveillance of antimicrobial resistance. J Infect Dis. 2020;222:1816–25. https://doi.org/10.1093/infdis/jiaa002
14. Demczuk W, Martin I, Sawatzky P, Allen V, Lefevbre B, Hoang L, et al. Equations to predict antimicrobial MICs in Neisseria gonorrhoeae using molecular antimicrobial resistance determinants. Antimicrob Agents Chemother. 2020;64:e02005–19. https://doi.org/10.1128/AAC.02005-19

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Association of Phylogenomic Relatedness between *Neisseria gonorrhoeae* Strains with Antimicrobial Resistance, Austria, 2016–2020

Appendix

Methods

**Whole-Genome Sequencing**

Genomic DNA isolation, WGS, assembly and contig filtering were performed as described previously (1). High-molecular-weight DNA was isolated from cultures using the MagAttract HMW DNA Kit (QIAGEN, Hilden, Germany), following the manufacturer’s protocol for Gram-negative bacteria. Ready-to-sequence libraries were obtained with NexteraXT kit following the manufacturer’s protocol (Illumina, CA, United States). Paired-end sequencing (2 × 300 bp) was performed on a MiSeq instrument as recommended by the manufacturer (Illumina). Raw reads were de novo assembled into a draft genome using SPAdes (version 3.11.1) (2). Contigs were filtered for a minimum coverage of 5 and minimum length of 200 bp. Sequencing quality was checked with FastQC. Sequencing generated 106,428 to 2,927,502 reads, a coverage of 12- to 272-fold (mean 76, 95% confidence interval [74.3–77.6]), a mean N50 of 38,513 (95% confidence interval [174–153,250]) and a mean contig length of 8,395 (95% confidence interval [208–23,184]).

**Core-Genome MLST (cgMLST)**

A local *N. gonorrhoeae* cgMLST scheme was generated with SeqSphere+ target definer tool (version 6.0.0, Ridom, Münster, Germany) (3). Strain MS11 was used as a seed genome (NCBI accession number NC_022240.1) and 47 complete *N. gonorrhoeae* genomes were used as query sequences (accession numbers NC_002946.2, NC_011035.1, NZ_CP012026.1, NZ_CP012027.1, NZ_CP012028.1, NZ_CP016015.1, NZ_CP016016.1, NZ_CP016017.1, ABZF00000000.1, ABZG00000000.1, ABZH00000000.1, ACIG00000000.1,
ADAA00000000.1, ABZJ00000000.2, ABZI00000000.1, ABZM00000000.1, ABZL00000000.1, ABZN00000000.1, ABZO00000000.1, ABZP00000000.1, ABZQ00000000.1, CQLK00000000.1, CQJM00000000.1, CQME00000000.1, CQJI00000000.1, CQIM00000000.1, CQHK00000000.1, CQLD00000000.1, CQN00000000.1, CQKW00000000.1, CQJY00000000.1, CQIY00000000.1, CQJB00000000.1, CQKU00000000.1, CQOV00000000.1, CQR00000000.1, CQJZ00000000.1, CQKM00000000.1, CQMI00000000.1, CM00000000.1, CQKB00000000.1, CQOT00000000.1, CQJ00000000.1, CHZN00000000.1, CFRU00000000.1, AKCG00000000.1, AKCH00000000.1), with default software parameters. A 1,524 loci cgMLST scheme and a 463 loci accessory target scheme were obtained, which were used in a previous publication (4).

Antimicrobial Resistance Genes Identifier, Adapted from PathogenWatch

Genotypic antibiotic resistance was investigated using allele libraries of 16S rDNA (coding for 16S ribosomal RNA), 23S rDNA (coding for 23S ribosomal RNA), blaTEM, ereA, ereB, ermA, ermB, ermC, ermF, folP, gyrA, macAB promoter, mefA, mtrC, mtrR, mtrR promoter, mtr mosaic, norM promoter, parC, parE, penA, ponA, porB1b, rplD, rplV, rpoB, rpoD, rpsE, rpsJ and tetM, based on the library of PathogenWatch in TOML format (version 0.0.14) (5). Each allele library was implemented in SeqSphere+ (Ridom) and used to search assembled genomes. Alleles were matched if they reached 99% alignment to reference sequences. Alleles with >90% identity to reference sequences but no match were defined as “new allele” and aligned with reference sequences to identify mutations. All 1,318 study isolates were searched for genetic AMR using this tool.

Data Analysis

Statistical analysis was performed using R version 4.0.4. A positive outcome was defined as resistance to azithromycin, cefixime, ciprofloxacin, tetracycline, or penicillin, reduced susceptibility to ceftriaxone, or positivity for cefinase. For time series analysis, thirteen-weeks moving averages of collection dates were calculated (R packages ISOweek (6), zoo (7)). The percentage of resistant isolates (or with reduced susceptibility to ceftriaxone/positive for cefinase) over time was plotted, and trends were calculated by linear regression.
For risk factor identification, odds ratios (OR) were calculated for each outcome using univariate analysis (package epitools (8)). Multivariate analysis consisted in logistic regression including several explanatory variables (function glm and package broom (9)). Only genes or mutations reported to induce AMR to a given antibiotic by the PathogenWatch tool (5) were considered as potential explanatory variables. Explanatory variables were progressively included in the model until the lowest Akaike information criterion was reached. Adjusted odds ratio (aOR) were calculated

**Data Visualization**

Isolates were characterized by seven loci MLST scheme (10), NG-MAST (11) and by an in-house cgMLST scheme using SeqSphere+ (Ridom). Minimum spanning trees (MST) were computed using the number of cgMLST allelic differences between 1,304 isolates (14 were excluded due to <90% cgMLST good targets). Neighbor-joining tree (NJT) of the cgMLST analysis was exported from SeqSphere+ (Ridom, Münster, Germany) and loaded into R to compute dendrograms (packages ggplot2 (12), ggpubr (13), ape (14), ggtree (15)). Histograms and boxplots were created with R packages ggplot2 (12), viridis (16), RColorBrewer (17) and scales (18).

**References**

1. Lepuschitz S, Sorschag S, Springer B, Allerberger F, Ruppitsch W. Draft genome sequence of carbapenemase-producing *Serratia marcescens* isolated from a patient with chronic obstructive pulmonary disease. Genome Announc. 2017;5:e01288–17. PubMed [https://doi.org/10.1128/genomeA.01288-17](https://doi.org/10.1128/genomeA.01288-17)

2. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, et al. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol. 2013;20:714–37. PubMed [https://doi.org/10.1089/cmb.2013.0084](https://doi.org/10.1089/cmb.2013.0084)

3. Ruppitsch W, Pietzka A, Prior K, Bletz S, Fernandez HL, Allerberger F, et al. Defining and evaluating a core genome multilocus sequence typing scheme for whole-genome sequence-based typing of *Listeria monocytogenes*. J Clin Microbiol. 2015;53:2869–76. PubMed [https://doi.org/10.1128/JCM.01193-15](https://doi.org/10.1128/JCM.01193-15)

4. Hirk S, Lepuschitz S, Cabal Rosel A, Huhulescu S, Blaschitz M, Stöger A, et al. Draft genome sequences of interpatient and intrapatient epidemiologically linked *Neisseria gonorrhoeae*
isolates. Genome Announc. 2018;6:e00319–18. PubMed
https://doi.org/10.1128/genomeA.00319-18

5. Sánchez-Busó L, Yeats CA, Taylor B, Goater RJ, Underwood A, Abudahab K, et al. A community-
driven resource for genomic epidemiology and antimicrobial resistance prediction of Neisseria
gonorrhoeae at Pathogenwatch. Genome Med. 2021;13:61. PubMed
https://doi.org/10.1186/s13073-021-00858-2

6. von Hatzfeld H. ISOweek: Week of the year and weekday according to ISO 8601. R package version
0.6–2. 2011 [cited 2022 Jan 8]. https://cran.r-project.org/web/packages/ISOweek/index.html

7. Zeileis A, Grothendieck G. zoo: S3 infrastructure for regular and irregular time series. [cited 2022 Jan
8]. J Stat Softw. 2005;14. https://doi.org/10.18637/jss.v014.i06

8. Aragon TJ. epitools: Epidemiology tools. R package version 0.5–10.1. 2020 [cited 2022 Jan 8].
https://cran.r-project.org/package=epitools

9. Robinson D, Hayes A, Couch S. broom: convert statistical objects into tidy tibbles. R package version
0.7.6. 2021 [cited 2022 Jan 8]. https://cran.r-project.org/web/packages/broom/index.html

10. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, et al. Multilocus sequence typing:
a portable approach to the identification of clones within populations of pathogenic
microorganisms. Proc Natl Acad Sci U S A. 1998;95:3140–5. PubMed
https://doi.org/10.1073/pnas.95.6.3140

11. Martin IM, Ison CA, Aanensen DM, Fenton KA, Spratt BG. Rapid sequence-based identification of
gonococcal transmission clusters in a large metropolitan area. J Infect Dis. 2004;189:1497–505. PubMed
https://doi.org/10.1086/383047

12. Wickman H. ggplot2: elegant graphics for data analysis. 2016 [cited 2022 Jan 8]. https://cran.r-
project.org/web/packages/ggplot2/index.html

13. Kassambara A. ggpubr: ‘ggplot2’ based publication ready plots. R package version 0.4.0. 2020 [cited
2022 Jan 8]. https://cran.r-project.org/web/packages/ggpubr/index.html

14. Paradis E, Schliep K. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in
R. Bioinformatics. 2019;35:526–8. PubMed https://doi.org/10.1093/bioinformatics/bty633

15. Yu G, Smith D, Zhu H, Guan Y, Tsan-Yuk Lam T. ggtree: an R package for visualization and
annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol
Evol. 2017;8:28–36. https://doi.org/10.1111/2041-210X.12628
16. Garnier S. viridis: default color maps from ‘matplotlib’. R package version 0.5.1. 2018 [cited 2022 Jan 8]. https://cran.r-project.org/web/packages/viridis/index.html

17. Neuwirth E. RColorBrewer: ColorBrewer palettes. R package version 1.1–2. 2014 [cited 2022 Jan 8]. https://cran.r-project.org/web/packages/RColorBrewer/index.html

18. Wickham H. scales: scale functions for visualization. R package version 1.1.1. 2020 [cited 2022 Jan 8]. https://cran.r-project.org/web/packages/scales/index.html
| AMR         | Resistant | #  | %   | Susceptible | #  | %   | OR (95%CI) | p.value |
|------------|-----------|----|-----|-------------|----|-----|------------|---------|
| Azithromycin | Cefixime  | 121| 0   | 0%          | 1180| 35 | 3%         | 0 [0-NA] | 0.06878 |
|              | Ceftriaxone | 122| 1   | 0.8%        | 1180| 32 | 2.7%       | 0.296 [0.04-2.19] | 0.3581 |
|              | Ciprofloxacin | 122| 88  | 72.1%       | 1179| 688| 58.4%      | 1.85 [1.22-2.79] | 0.0035 |
|              | Tetracycline | 121| 86  | 71.1%       | 1077| 472| 43.8%      | 3.15 [2.09-4.75] | 0      |
|              | Penicillin | 122| 2   | 1.6%        | 1180| 202| 17.1%      | 0.081 [0.02-0.329] | 0      |
|              | Cefinase  | 121| 1   | 0.8%        | 1135| 181| 15.9%      | 0.044 [0.006-0.316] | 0      |
| Ceftriaxone  | Azithromycin | 35 | 0   | 0%          | 1266| 121| 9.6%       | 0 [0-NA] | 0.06878 |
|              | Ceftriaxone | 35 | 16  | 45.7%       | 1276| 17 | 1.3%       | 62.4 [27.5-142] | 0      |
|              | Ciprofloxacin | 35 | 35  | 100%        | 1275| 746| 58.5%      | Inf [NA-Inf] | 0      |
|              | Tetracycline | 27 | 19  | 70.4%       | 1180| 542| 45.9%      | 2.8 [1.21-6.44] | 0.01761 |
|              | Penicillin | 35 | 23  | 65.7%       | 1276| 182| 14.3%      | 11.5 [5.63-23.6] | 0      |
|              | Cefinase  | 35 | 1   | 2.9%        | 1230| 182| 14.8%      | 0.169 [0.023-1.25] | 0.04921 |
| Ciprofloxacin | Azithromycin | 33 | 1   | 3%          | 1269| 121| 9.5%       | 0.296 [0.04-2.19] | 0.3581 |
|              | Cefixime  | 33 | 16  | 48.5%       | 1278| 19 | 1.5%       | 62.4 [27.5-142] | 0      |
|              | Ceftriaxone | 33 | 32  | 97%         | 1278| 750| 58.7%      | 22.5 [3.07-165] | 0      |
|              | Tetracycline | 25 | 19  | 76%         | 1183| 543| 45.9%      | 3.73 [1.48-9.41] | 0.00375 |
|              | Penicillin | 25 | 22  | 66.7%       | 1279| 183| 14.3%      | 12 [5.71-25.1] | 0      |
| Penicillin   | Azithromycin | 776| 88  | 11.3%       | 525 | 34 | 6.5%       | 1.85 [1.22-2.79] | 0.0035 |
|              | Cefixime  | 781| 35  | 4.5%        | 529 | 0  | 0%         | Inf [NA-Inf] | 0      |
|              | Ceftriaxone | 782| 32  | 4.1%        | 529 | 1  | 0.2%       | 22.5 [3.07-165] | 0      |
|              | Tetracycline | 714| 480 | 67.2%       | 494 | 82 | 16.6%      | 10.3 [7.76-13.7] | 0      |
| Penicillin   | Ceftriaxone | 782| 200 | 25.6%       | 529 | 4  | 0.8%       | 45.1 [16.6-122] | 0      |
|              | Cefixime  | 748| 177 | 23.7%       | 517 | 5  | 1%         | 31.7 [12.9-77.8] | 0      |
| Tetracycline | Azithromycin | 558| 86  | 15.4%       | 640 | 35 | 5.5%       | 3.15 [2.09-4.75] | 0      |
|              | Cefixime  | 561| 19  | 3.4%        | 646 | 8  | 1.2%       | 2.8 [1.21-6.44] | 0.01761 |
|              | Ceftriaxone | 562| 19  | 3.4%        | 646 | 6  | 0.9%       | 3.73 [1.48-9.41] | 0.00375 |
| Ciprofloxacin | Azithromycin | 562| 480 | 85.4%       | 646 | 234| 36.2%      | 10.3 [7.76-13.7] | 0      |
|              | Cefixime  | 562| 141 | 25.1%       | 646 | 33 | 5.1%       | 6.22 [4.17-9.27] | 0      |
|              | Penicillin | 545| 143 | 26.2%       | 617 | 19 | 3.1%       | 11.2 [6.83-18.4] | 0      |
| Penicillin   | Azithromycin | 204| 2   | 1%          | 1098| 120| 10.9%      | 0.081 [0.02-0.329] | 0      |
|              | Cefixime  | 205| 23  | 11.2%       | 1106| 12 | 1.1%       | 11.5 [5.63-23.6] | 0      |
|              | Ceftriaxone | 205| 22  | 10.7%       | 1107| 11 | 1%         | 12 [5.71-25.1] | 0      |
| Ciprofloxacin | Azithromycin | 204| 200 | 98%         | 1107| 582| 52.6%      | 45.1 [16.6-122] | 0      |
|              | Cefixime  | 174| 141 | 81%         | 1034| 421| 40.7%      | 6.22 [4.17-9.27] | 0      |
|              | Tetracycline | 194| 150 | 77.3%       | 1072| 33 | 3.1%       | 107 [66.2-174] | 0      |
| Cefinase     | Azithromycin | 182| 1   | 0.5%        | 1074| 120| 11.2%      | 0.044 [0.006-0.316] | 0      |
|              | Cefixime  | 183| 1   | 0.5%        | 1082| 34 | 3.1%       | 0.169 [0.023-1.25] | 0.04921 |
|              | Ceftriaxone | 183| 2   | 1.1%        | 1083| 27 | 2.5%       | 0.432 [0.102-1.83] | 0.41838 |
| Ciprofloxacin | Azithromycin | 182| 177 | 97.3%       | 1083| 571| 52.7%      | 31.7 [12.9-77.8] | 0      |
|              | Cefixime  | 162| 143 | 88.3%       | 1000| 402| 40.2%      | 11.2 [6.83-18.4] | 0      |
|              | Tetracycline | 183| 150 | 82%         | 1083| 44 | 4.1%       | 107 [66.2-174] | 0      |

* A positive outcome was defined as resistance to azithromycin, cefixime, tetracycline, and penicillin, reduced susceptibility to ceftriaxone and positivity for cefinase. For each variable, number of isolates (#), total number of isolates and frequency (%) are indicated for resistant (or with reduced susceptibility/positive) and susceptible (or negative) isolates. Odds ratio (OR) and 95% confidence interval were calculated by univariate analysis and association was tested with Fisher exact test.
Appendix Table 2. Genes and point mutations associated with antimicrobial resistance in *N. gonorrhoeae* isolates (N = 1,318). For each gene, number of isolates (#), total number of isolates for which the gene was found (tot) and frequency (%) are indicated.

| Gene         | Variant               | #     | %     |
|--------------|-----------------------|-------|-------|
| 16S_rDNA     | C1450                 | 639   | 48.5% |
|              | none                  | 545   | 41.4% |
|              | NA                    | 134   | 10.2% |
| 23S_rDNA     | C2597                 | 3     | 0.2%  |
|              | C2597.C265            | 1     | 0.1%  |
|              | C265                  | 123   | 9.3%  |
|              | none                  | 1124  | 85.3% |
|              | NA                    | 67    | 5.1%  |
| blaTEM       | not found             | 1185  | 89.9% |
|              | found                 | 133   | 10.1% |
| ereA         | not found             | 1318  | 100%  |
|              | found                 | 0     | 0%    |
| ereB         | not found             | 1318  | 100%  |
|              | found                 | 0     | 0%    |
| emmA         | not found             | 1318  | 100%  |
|              | found                 | 0     | 0%    |
| emmB         | not found             | 1318  | 100%  |
|              | found                 | 0     | 0%    |
| emmC         | not found             | 1318  | 100%  |
|              | found                 | 0     | 0%    |
| emmF         | not found             | 1314  | 99.7% |
|              | found                 | 4     | 0.3%  |
| folP         | R228                  | 1081  | 0.82  |
|              | none                  | 229   | 17.4% |
|              | NA                    | 8     | 0.6%  |
| gyrA         | D95                   | 484   | 36.7% |
|              | D95.S91               | 291   | 22.1% |
|              | none                  | 529   | 40.1% |
|              | NA                    | 14    | 1.1%  |
| macAB_promotor | mut-10               | 129   | 9.8%  |
|              | none                  | 1182  | 89.7% |
|              | NA                    | 7     | 0.5%  |
| mtaA         | not found             | 1318  | 100%  |
|              | found                 | 0     | 0%    |
| mtr_mosaic   | not found             | 1146  | 86.9% |
|              | found                 | 172   | 13.1% |
| mtrC         | frameshift            | 23    | 1.7%  |
|              | none                  | 1282  | 97.3% |
|              | NA                    | 13    | 1.0%  |
| mtrR         | found                 | 1258  | 95.4% |
|              | A39                   | 337   | 25.6% |
|              | A39.G45               | 48    | 3.6%  |
|              | frameshift            | 126   | 9.6%  |
|              | G45                   | 160   | 12.1% |
|              | none                  | 637   | 48.3% |
|              | NA                    | 10    | 0.8%  |
| mtrR_promoter | C187G                | 7     | 0.5%  |
|              | del-35                | 302   | 22.9% |
|              | ins266A+ins253G       | 88    | 6.7%  |
|              | none                  | 731   | 55.5% |
|              | NA                    | 190   | 14.4% |
| norM_promoter | ins211               | 62    | 4.7%  |
|              | ins211.ins250         | 124   | 9.4%  |
|              | ins250                | 5     | 0.4%  |
|              | none                  | 1117  | 84.7% |
|              | NA                    | 10    | 0.8%  |
| parC         | D86N                  | 284   | 21.5% |
|              | E91G/Q                | 80    | 6.1%  |
|              | E91G/Q.S87I           | 12    | 0.9%  |
|              | E91G/Q.S87N           | 20    | 1.5%  |
|              | E91K.S87N             | 25    | 1.9%  |
|              | S87I                  | 1     | 0.1%  |
|              | S87N                  | 29    | 2.2%  |
|              | S87N.S88P             | 2     | 0.2%  |
|              | S87R                  | 198   | 0.15  |
|              | S87R.S88P             | 103   | 7.8%  |
|              | S88P                  | 21    | 1.6%  |
| Gene | Variant | #   | %    |
|------|---------|-----|------|
|      | none    | 529 | 40.1%|
|      | NA      | 14  | 1.1% |
| penA | A501T/V | 1   | 0.1% |
|      | A501T/V.ins346D | 63  | 4.8% |
|      | A501T/V.ins346D.P551S/L | 94  | 7.1% |
|      | G545S.I312M+V316T.I312M+V316T | 304 | 23.1%|
|      | I312M+V316T | 2   | 0.2% |
|      | I312M+V316T.P551S/L | 1   | 0.1% |
|      | ins346D  | 706 | 53.6%|
|      | ins346D.P551S/L | 16  | 1.2% |
|      | none     | 119 | 0.09 |
|      | NA       | 12  | 0.9% |
|      | A501T/V  | 561 | 42.6%|
|      | none     | 738 | 0.56 |
|      | NA       | 19  | 1.4% |
|      | A121     | 182 | 12.3%|
|      | A121.G120 | 392 | 29.7%|
|      | G120     | 33  | 2.5% |
|      | none     | 579 | 43.9%|
|      | NA       | 152 | 11.5%|
|      | G68      | 18  | 1.4% |
|      | G70      | 40  | 0.03 |
|      | none     | 1252| 0.95 |
|      | NA       | 8   | 0.6% |
|      | D11      | 141 | 10.7%|
|      | none     | 1170| 88.8%|
|      | NA       | 7   | 0.5% |
|      | V57      | 961 | 72.9%|
|      | none     | 342 | 25.9%|
|      | NA       | 15  | 1.1% |
| tetM | not found | 1061| 80.5%|
|      | found    | 257 | 19.5%|
| All  |         | 1318| 100% |
| Amr          | Patient data variable | Category | Resistant | Susceptible | Univariate analysis | multivariate analysis |
|-------------|-----------------------|----------|-----------|-------------|---------------------|----------------------|
|             |                       |          | Total #   | %           | Total #             | OR [95%CI]           |
|             |                       |          |           |             |                     | p.value              |
|             |                       |          |           |             |                     | OR [95%CI]           |
|             |                       |          |           |             |                     | p.value              |
| Azithromycin|                       |          | C265T     | 115         | 3                  | 1122                 | 120                  |
|             | macAB_promotor        | mut-10   | 120       | 60.7%       | 175                 | 48                   |
|             | mtrR_promoter         | del-35   | 121       | 78.3%       | 1101                | 293                  |
|             |                       | found     | 122       | 103         | 1180                | 66                   |
|             |                       | mtr-35    | A39T      | 120         | 11.9%              | 172                  | 370                  |
|             |                       | frameshift| 120       | 0.8%        | 117                 | 121                  |
|             | mtrR                 | G45D/S    | 120       | 3            | 25.5%              | 112                  | 204                  |
|             | Ceftriaxone           | mtrR_promoter | del-35  | 35         | 0                   | 1088                 | 302                  |
|             |                       | mtrR      | A39T      | 35         | 0                   | 1266                 | 383                  |
|             |                       | penA      | G45D/S    | 35         | 29                  | 122                   | 179                  |
|             |                       | G545S     | 35         | 35          | 100%                | 1264                 | 268                  |
|             |                       | penA      | A501T/V   | 32         | 5                   | 16.6%                | 1268                 | 153                  |
|             |                       | G545S     | 32         | 26          | 81.2%              | 1268                 | 277                  |
|             |                       | penA      | I312M+V316T| 32        | 26                  | 81.2%                | 1268                 | 280                  |
|             |                       | ins346D   | 32         | 5           | 15.6%              | 1268                 | 869                  |
|             | Ciprofloxacin         | gyrA      | D95N/G/A/Y| 772       | 1267                | 99.4%                | 525                  |
|             |                       | S91F/T    | 772       | 1266        | 99.4%              | 525                  |
|             |                       | norM_promoter | ins211A | 775       | 116                 | 20.8%                | 526                  |
|             |                       | ins250T   | 775       | 117         | 15.1%              | 526                  |
|             |                       | parC      | D86N      | 774       | 252                 | 32.6%                | 523                  |
|             |                       | E91G/Q    | 774       | 91          | 2.5%               | 523                  |
|             |                       | S87N      | 774       | 75          | 9.7%               | 523                  |
|             | Tetracycline          | mtrR_promoter | del-35 | 437       | 128                 | 29.3%                | 584                  |
|             |                       | mtrR      | A39T      | 560       | 102                 | 19.6%                | 639                  |
|             |                       | G545D/S   | 560       | 110         | 19.6%              | 639                  |
|             |                       | rpsJ      | V57M      | 555       | 552                 | 99.5%                | 639                  |
|             |                       | tetM      | found     | 562       | 223                 | 39.7%                | 646                  |
|             | Penicillin            | blaTEM    | found     | 205       | 100                 | 48.8%                | 1107                 |
|             |                       | mtrR_promoter | del-35 | 203       | 30                   | 14.8%                | 920                  |
|             |                       | ins266A+ins253 | 203     | 20         | 9.9%                | 920                  |
|             | Cefinase              | blaTEM    | found     | 183       | 118                 | 64.5%                | 1083                 |

*Appendix Table 3. Genetic risk factors associated with resistant *N. gonorrhoeae* isolates (N = 1,318)*

| Amr          | Category | OR [95%CI]           |
|-------------|----------|----------------------|
|             |          | 0.224 [0.070–0.715]  |
|             |          | 0.00294              |
|             |          | 47 [29.1–75.7]       |
|             |          | 0                    |
|             |          | 27.7 [1.3–231]       |
|             |          | 0.00566              |
|             |          | 3.86 [1.22–12.3]     |
|             |          | 0.02107              |
|             |          | 2.59 [0.594–11.2]    |
|             |          | 0.18732              |
|             |          | 88.7 [51.3–153]      |
|             |          | 0                    |
|             |          | 0.219 [0.116–0.412]  |
|             |          | 0.00012              |
|             |          | 0.122 [0.038–0.387]  |
|             |          | 1.00E-06             |
|             |          | 1.54e-07 [NA-1.63e+37]|
|             |          | 0.98996              |
|             |          | 0                    |
|             |          | 0                    |
|             |          | 29.4 [12.7–71.7]     |
|             |          | 0                    |
|             |          | 2.27 [0.875–6.71]    |
|             |          | 0.10866              |
|             |          | 1.35 [0.512–3.56]    |
|             |          | 0.57969              |
|             |          | 73.9 [6.3–317e+03]   |
|             |          | 0.00421              |
|             |          | 15.5 [6.32–38]       |
|             |          | 0                    |
|             |          | 16.2 [2.95–369]      |
|             |          | 0.01321              |
|             |          | 0.085 [0.033–0.222]  |
|             |          | 0.0723 [0.00231–2.182]| 0.09253
|             |          | 1.66e+04 [4.46e+03–| 0.756e+03 [2.33e+03–| 0.866e-27
|             |          | 5.54e+04              |
|             |          | 3.11e+04              |
|             |          | 315 [44.1–225e+03]   |
|             |          | 0                    |
|             |          | 5.49 [3.52–8.56]     |
|             |          | 0.00367              |
|             |          | 8.11 [1.04–53.2]     |
|             |          | 0.02678              |
|             |          | 8.22 [5.49–12.3]     |
|             |          | 0.0268               |
|             |          | 4.5 [0.814–26.8]     |
|             |          | 0.00266              |
|             |          | 334 [104–21.4e+03]   |
|             |          | 0.144e-01             |
|             |          | 67.9 [35.2–139]      |
|             |          | 0.00035              |
|             |          | 0.0187               |
|             |          | 0.01352              |
|             |          | 0.00033              |
|             |          | 0.02552              |
|             |          | 0.00033              |
|             |          | 0.00033              |
|             |          | 0.01352              |
|             |          | 0.00033              |
|             |          | 0.02552              |
|             |          | 0.00033              |
|             |          | 0.00033              |
|             |          | 0.00033              |
|             |          | 0.00033              |
|             |          | 0.00033              |
|             |          | 0.00033              |
|             |          | 0.00033              |

- G: OR [95%CI]
- R: p-value
- C: OR [95%CI]
- F: p-value
- Z: OR [95%CI]
- P: p-value
- M: OR [95%CI]
- T: p-value
| Variable | Category | Resistant | Susceptible | Univariate analysis | multivariate analysis |
|----------|----------|-----------|-------------|---------------------|----------------------|
|          | Tot | # | % | Tot | # | % | OR [95%CI] | p.value | OR [95%CI] | p.value |
| mtrR_promoter | del-35 | 180 | 25 | 13.9% | 898 | 267 | 29.7% | 0.381 [0.244–0.595] | 6.00E-06 | 0.0892 [0.0248–0.277] | 8.56E+09 |
| | ins266A+ins253 | 180 | 21 | 11.7% | 898 | 64 | 7.1% | 1.72 [1.02–2.9] | 0.04805 | 5.77 [2.23–14.2] | 0.00017 |
| mtrR | A39T | 182 | 76 | 41.8% | 1074 | 300 | 27.9% | 1.85 [1.02–2.9] | 0.04805 | 5.77 [2.23–14.2] | 0.00017 |
| | frameshift | 182 | 35 | 19.2% | 1074 | 88 | 8.2% | 2.67 [1.74–4.09] | 2.00E-05 | 3.56 [14–97.4] | 3.43E+01 |
| penA | A501T/V | 181 | 56 | 30.9% | 1073 | 97 | 9% | 4.51 [3.09–6.58] | 0 | 3.56 [14–97.4] | 3.43E+01 |
| | G54S | 181 | 29 | 16% | 1073 | 253 | 23.8% | 0.618 [0.406–0.942] | 0.02655 | 0.618 [0.406–0.942] | 0.02655 |
| | I312M+V316T | 181 | 29 | 16% | 1073 | 235 | 22.8% | 0.612 [0.402–0.933] | 0.02117 | 0.612 [0.402–0.933] | 0.02117 |
| | ins346D | 181 | 150 | 82.9% | 1073 | 706 | 65.8% | 2.52 [1.68–3.78] | 3.00E-06 | 3.49 [1.66–7.57] | 0.00116 |
| | P551S/L | 181 | 8 | 4.4% | 1073 | 97 | 9% | 0.465 [0.222–0.974] | 0.04125 | 0.107 [0.0287–0.378] | 0.00058 |
| porB1b | A121D/N/S/G/V | 147 | 52 | 35.4% | 973 | 472 | 48.5% | 0.581 [0.405–0.833] | 0.00334 | 0.581 [0.405–0.833] | 0.00334 |
| | G120K/N/D/Q/R | 147 | 60 | 40.8% | 973 | 336 | 34.5% | 1.31 [0.917–1.86] | 0.13973 | 4.56 [2.17–9.67] | 6.61E+09 |

*A positive outcome was defined as resistance to azithromycin, cefixime, tetracycline and penicillin, reduced susceptibility to ceftriaxone and positivity for cefinase. For each variable, number of isolates (#), total number of isolates and frequency (%) are indicated for resistant (or with reduced susceptibility/positive) and susceptible (or negative) isolates. For univariate analysis, odds ratio (OR) and 95% confidence interval were calculated and association was tested with Fisher exact test. For multivariate analysis, variables with significant association in univariate analysis were included in a logistic regression model. Adjusted odds ratio (aOR), 95% confidence interval and p-value were calculated for the model with the lowest Akaike information criterion.*