

Genomic Epidemiology of Azithromycin-Nonsusceptible *Neisseria gonorrhoeae*, Argentina, 2005–2019

Appendix

Appendix Methods

Antimicrobial Susceptibility Testing

We subcultured *N. gonorrhoeae* isolates on Difco GC medium agar base (Becton, Dickinson and Company, <https://www.bd.com>) supplemented with 1% Britalex enrichment supplement (Laboratorios Britania S.A., <https://www.britanialab.com>) for 18–24 h at 35°C in a humid 5% carbon dioxide–enriched atmosphere before conducting antimicrobial susceptibility testing. We determined the MICs of azithromycin, ceftriaxone, cefixime, ciprofloxacin, spectinomycin, benzylpenicillin, tetracycline, and gentamicin (MilliporeSigma, <https://www.sigmaaldrich.com>) using the agar dilution method (Reference 1 in Appendix). We interpreted the MICs using CLSI breakpoints (15), except for gentamicin, for which we used previously published interpretive criteria (Reference 2 in Appendix). We used the *N. gonorrhoeae* strain ATCC 49226 and 8 WHO reference strains documented in 2008 for quality control (15, Reference 3 in Appendix).

Whole-Genome Sequencing

We extracted genomic DNA using QIAamp DNA Mini Kit (QIAGEN, <https://www.qiagen.com>) according to the manufacturer's instructions. We determined DNA concentration using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, <https://www.thermofisher.com>) and stored samples at –20°C. We conducted WGS on all isolates using the Nextera XT DNA library preparation kit and the MiSeq Platform (Illumina, <https://www.illumina.com>) according to the manufacturer's instructions. We assessed the quality of the sequences using FastQC version 0.11.9 (Babraham Institute,

<http://www.bioinformatics.babraham.ac.uk>) and identified contaminants using Kraken 2 version 2.08 (Johns Hopkins University, <http://ccb.jhu.edu>). We assembled de novo reads using Unicycler version 0.4.8, which is based on SPAdes version 3.13.0 (Reference 4 in Appendix), and assessed assembly quality using Quast version 5.0.2 (Reference 5 in Appendix). On average, the numbers of contigs was 103 and the N50 contig length (i.e., the length for which half of the bases of a draft genome are situated in contigs of that length or longer) was 48,458 bp.

Additional References

1. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard-eleventh edition. Wayne (PA): The Institute; 2019.
2. Gianecini R, Oviedo C, Irazu L, Rodríguez M, Galarza P; GASSP-AR Working Group. Comparison of disk diffusion and agar dilution methods for gentamicin susceptibility testing of *Neisseria gonorrhoeae*. *Diagn Microbiol Infect Dis*. 2018;91:299–304. [PubMed](#)
<https://doi.org/10.1016/j.diagmicrobio.2018.03.005>
3. Unemo M, Fasth O, Fredlund H, Limnios A, Tapsall J. Phenotypic and genetic characterization of the 2008 WHO *Neisseria gonorrhoeae* reference strain panel intended for global quality assurance and quality control of gonococcal antimicrobial resistance surveillance for public health purposes. *J Antimicrob Chemother*. 2009;63:1142–51. [PubMed](#) <https://doi.org/10.1093/jac/dkp098>
4. Antipov D, Korobeynikov A, McLean JS, Pevzner PA. hybridSPAdes: an algorithm for hybrid assembly of short and long reads. *Bioinformatics*. 2016;32:1009–15. [PubMed](#)
<https://doi.org/10.1093/bioinformatics/btv688>
5. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*. 2013;29:1072–5.

N. gonorrhoeae FA1090	MAKFFIDRPI	PAWVISIFII	AAGIFGIKSL	PVSQYPSVAA	PTITLHAIYP	GASAOVMEGS	VLSVIERMNM	GV EGLDYMST	SADSSGSGSV	SLTFTPTDTE	NLAQVEVQNK	LSEVLSTLPA	120
CDC2	120
CCITS-50 (Clade 2)	120
CGGS0402	120
CCITS-20 (n=1)	120
CCITS-60 (n=2)	120
N. gonorrhoeae FA1090	TVQQYGVTVS	KARSNFLMIV	MLSSDVQSTE	EMNDYQRNV	VPELQRIEIV	GVRLFGAQR	AMRWDPKK	LQNYLSPAD	VGSALSQNI	QISAGSIGSL	PAVGGQVTA	TVTAQGLQGT	240
CDC2	240
CCITS-50 (Clade 2)	240
CGGS0402	240
CCITS-20 (n=1)	240
CCITS-60 (n=2)	240
N. gonorrhoeae FA1090	AEEFGVILR	ANTDGSNIYL	KDVAKVGLGM	EDYSSSTRLN	GVNTTGMAVM	LSNSGNAMAT	AKAVKERLAV	LEKYFPOGMS	WKTPTYDTSKF	VEISIEKVIH	TLIEAMVLFV	VVMYLFQNI	360
CDC2	360
CCITS-50 (Clade 2)	360
CGGS0402	360
CCITS-20 (n=1)	360
CCITS-60 (n=2)	360
N. gonorrhoeae FA1090	RYLLIPTIV	PISLLGGFAF	ISYMGMSINV	LTMFAMLVI	GIUVDQIAIV	VENVERIMAG	EGLPPKEATK	KAMGIGSIV	IGITAVLISV	FVPLAMFSGA	AGNIYKQFAL	TMASSIAFSA	480
CDC2	480
CCITS-50 (Clade 2)	480
CGGS0402	480
CCITS-20 (n=1)	480
CCITS-60 (n=2)	480
N. gonorrhoeae FA1090	FLALTLPAL	CATMLKTIK	GHHEKGGFF	GWFNKFDVW	THGYEGRVAK	VLKTKFRMV	VYIGLAVGV	FLFMRLPTSF	LPTEDOGFVM	VSVLQAGAT	KERTDATALQ	VTLAKSIEPE	600
CDC2	600
CCITS-50 (Clade 2)	600
CGGS0402	600
CCITS-20 (n=1)	600
CCITS-60 (n=2)	600
N. gonorrhoeae FA1090	IENIITVSGF	SFSGGQDMA	MGEAIIKQDN	ERTASGSDAV	AVAGKLTGM	MGLTKDGFGI	SVVPPPILEL	GNSSGLSINL	QDRNNTGHTA	LLAKRNELIQ	KMRASGLFDP	STVRAGGLEL	720
CDC2	720
CCITS-50 (Clade 2)	720
CGGS0402	720
CCITS-20 (n=1)	720
CCITS-60 (n=2)	720
N. gonorrhoeae FA1090	SPQLKIDINR	AAAAAGGISF	ADIRITALASA	LSSSYVDFP	NOGRLQRVMV	QAEEDARMQP	ADIILNLTVPN	KSGVAVPLST	IATVSWKNGT	EQSVRFNGYP	SMKLSASPAT	GVSTGQAMAA	840
CDC2	840
CCITS-50 (Clade 2)	840
CGGS0402	840
CCITS-20 (n=1)	840
CCITS-60 (n=2)	840
N. gonorrhoeae FA1090	VQKQVDELGG	GYSFEWGGQS	REEAKGGSQT	LILYGLAVAA	VFLVLAALYE	SWSIPLAVIL	VIPLGLIGAA	AGVTGRNLFE	GLLGSVPSPA	NDIYFVGVFV	TVMGLSAKNA	ILIIIEFAKDL	960
CDC2	960
CCITS-50 (Clade 2)	960
CGGS0402	960
CCITS-20 (n=1)	960
CCITS-60 (n=2)	960
N. gonorrhoeae FA1090	QAQKSAVEA	ALEAARLRF	PIIMTSFAP	LGVVPLYIAA	GASSASQRAI	GTVVFGMLV	GTLLSVFLVP	LFPVVVRKFF	KETAHEHMA	VRHASKAGIT	GSDDKQY*	1068	
CDC2	1068	
CCITS-50 (Clade 2)	1068	
CGGS0402	1068	
CCITS-20 (n=1)	1068	
CCITS-60 (n=2)	1068	

Appendix Figure. Amino acid sequences of mosaic MtrD loci in *N. gonorrhoeae* isolates, Argentina, 2005–2019. Amino acid sequences are aligned to the wildtype sequence of *N. gonorrhoeae* FA1090 (GenBank accession no. AE004969) and previously described mosaic MtrD sequences from CDC-2 and CGGS0834 strains (44,45). Dashes indicate amino acid residues identical to those of FA1090.