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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

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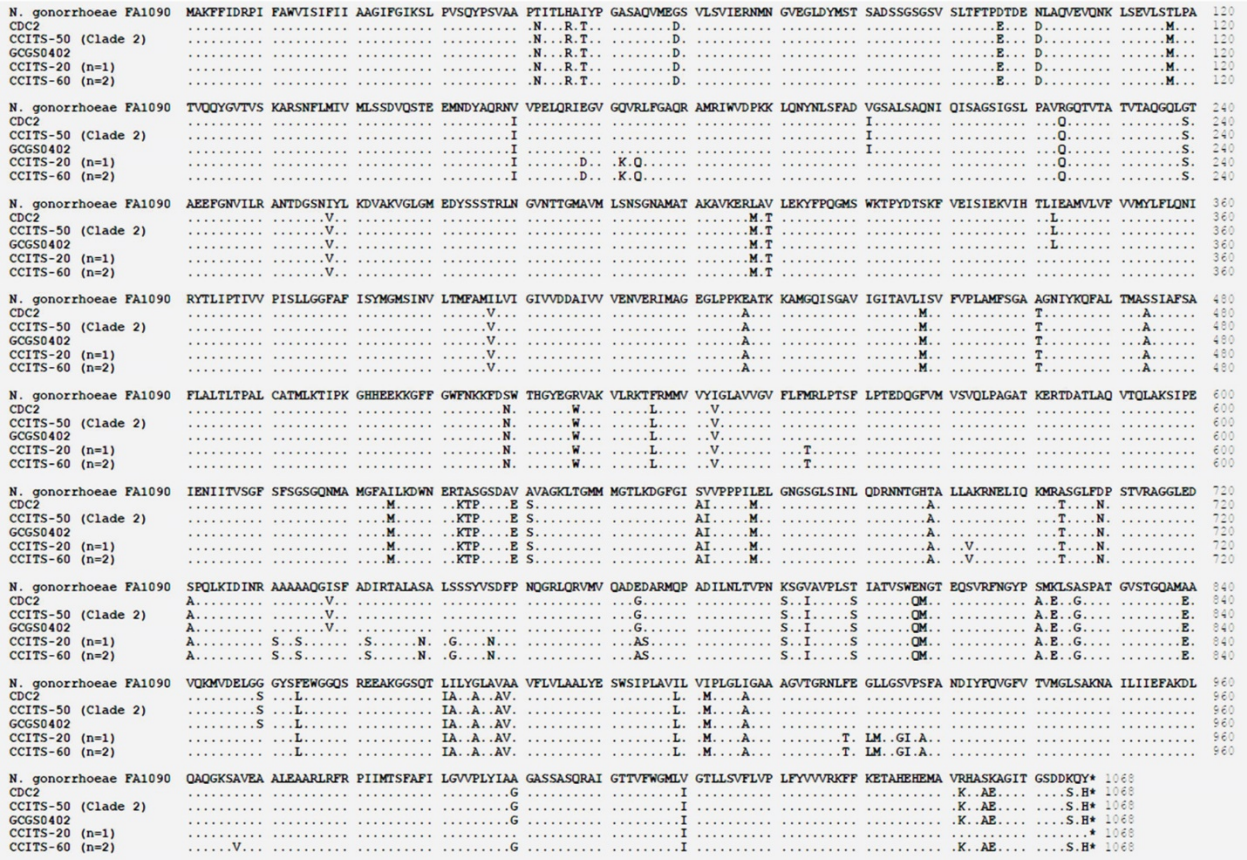

Appendix Figure. Amino acid sequences of mosaic MtrD loci in *N. gonorrhoeae* isolates, Argentina, 2005–2019. Amino acid sequences are aligned to the wildtype sequence of *N. gonorrhoeae* FA1090 (GenBank accession no. AE004969) and previously described mosaic MtrD sequences from CDC-2 and GCGS0834 strains (*44*,*45*). Dashes indicate amino acid residues identical to those of FA1090.