AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

Whole-Genome Sequencing of a Large Panel of Contemporary *Neisseria gonorrhoeae* Clinical Isolates Indicates that a Wild-Type *mtrA* Gene Is Common: Implications for Inducible Antimicrobial Resistance

Eshaw Vidyaprakash,[a] A. Jeanine Abrams,[a] William M. Shafer,[b,c] David L. Trees[a]

Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Atlanta, Georgia, USA[a]; Department of Microbiology and Immunology and the Emory Antibiotic Resistance Center, Emory University School of Medicine, Atlanta, Georgia, USA[b]; The Laboratories of Bacterial Pathogenesis, Medical Research Service, VA Medical Center (Atlanta), Decatur, Georgia, USA[c]

KEYWORDS MtrCDE efflux pump, *Neisseria gonorrhoeae*, *mtrA*

Previous studies (1) have shown that the expression of the *mtrCDE* efflux pump operon is inducible at the transcriptional level in gonococci during incubation in sublethal concentrations of the nonionic detergent Triton X-100, which along with antibiotics, bile salts, progesterone, and antimicrobial peptides is a substrate of the MtrCDE efflux pump (2, 8). Inducible expression of *mtrCDE* requires the DNA-binding action of MtrA (1). Under inducing conditions, MtrA activation of *mtrCDE* results in inducible antimicrobial resistance of gonococci to antimicrobials and increases bacterial fitness during experimental lower genital tract infection of female mice (3). MtrA binds upstream of the promoter used for transcription of *mtrCDE*, and this is increased in the presence of an inducer (4). MtrA was first identified in gonococci by homology to AraC/XlyS-like proteins (MarR and SoxS) of *Escherichia coli* (1). An earlier (1) examination of the FA1090 strain whole-genome sequence (WGS) revealed an open reading frame that encoded a truncated protein with homology to the N-terminal region of AraC proteins (e.g., SoxS, MarA, and AraC). Subsequent amplification of genomic DNA by PCR and sequencing of this region from *Neisseria gonorrhoeae* strain FA19 revealed that it had an 11-bp insertion (5′-GTCGGTACGGC-3′), compared to FA1090, encoding a full-length MtrA protein (GenBank accession no. AF128627); only 1 of 3 additional isolates had this insertion. Loss of MtrA, due to the aforementioned 11-bp deletion, rendered gonococci incapable of induction to higher levels of resistance to MtrCDE efflux substrates, and it had a negative impact on *in vivo* fitness (1, 3).

To gain insight regarding the status in contemporary gonococcal clinical isolates, we interrogated previously described (4) WGS from 922 isolates, including 804 isolates collected through the Gonococcal Isolate Surveillance Project (GISP) and 118 isolates collected from Rio de Janeiro, Brazil. To determine if the presence of a mutant *mtrA* is a common property of gonococci, bioinformatic analyses were employed to detect this 11-bp deletion in WGS. Briefly, paired-end WGS were run on an Illumina HiSeq 2500 sequencer. During preprocessing, the reads were cleaned and checked for quality using CLC Genomics Workbench software (Genomics Workbench 8.0.3; https://www.qiagenbioinformatics.com/), and *de novo* assembly and assessment (e.g., the number of contigs and N_{50} values) were conducted using the SPAdes assembler (5) and the

Accepted manuscript posted online 13 February 2017

Citation Vidyaprakash E, Abrams AJ, Shafer WM, Trees DL. 2017. Whole-genome sequencing of a large panel of contemporary *Neisseria gonorrhoeae* clinical isolates indicates that a wild-type *mtrA* gene is common: implications for inducible antimicrobial resistance. Antimicrob Agents Chemother 61:e00262-17. https://doi.org/10.1128/AAC.00262-17.

Address correspondence to Eshaw Vidyaprakash, kyi7@cdc.gov.

Quality Assessment Tool for Genome Assemblies (6), respectively. The SPAdes assembler uses a multi-k-value default, so it is usually unnecessary to add a k-mer size. However, 10 sequences exhibited a relatively higher number of contigs, so kmergenie (7) was used to determine an optimal k-mer size. The k-mer value was utilized as an input parameter for SPAdes analyses, resulting in reduced contig numbers after assembly. The complete *mtrA* sequence was identified in each assembly, but the 11-bp deletion (mutant) was not detected in the GISP samples, and it was absent in all but 4 of the 118 Brazilian isolates. Therefore, the presence of the wild-type *mtrA* is common among gonococci, and the majority of strains likely have the capacity to display inducible antimicrobial resistance through the MtrCDE efflux pump.

ACKNOWLEDGMENTS

W.M.S. is supported by NIH grant R37 AI21150-32 and a Senior Research Career Scientist Award from the Biomedical Laboratory Research and Development Service of the U.S. Department of Veterans Affairs. This work was funded by the Centers for Disease Control and Prevention (CDC) and the Office of Advanced Molecular Detection at CDC. The contents of this article are solely the responsibility of the authors and do not necessarily reflect the official views of the National Institutes of Health, the U.S. Department of Veterans Affairs, or the CDC.

The authors wish to acknowledge GISP principal investigators Robert Kirkcaldy and John Papp and Brazilian collaborators Ana Paula Ramalho da Costa Lourenço and Raquel Regina Bonelli.

We have no competing interests to declare.

REFERENCES

1. Rouquette C, Harmon J, Shaefer W. 1999. Induction of the *mtrCDE*-encoded efflux pump system of *Neisseria gonorrhoeae* requires MtrA, an AraC-like protein. Mol Microbiol 33:651–658. https://doi.org/10.1046/j.1365-2958.1999.01517.x.
2. Zalucki YM, Mercante AD, Cloward JM, Ohneck EA, Kandler JL, Goytia M, Johnson PJT, Shafer WM. 2013. Function and regulation of *Neisseria gonorrhoeae* efflux pumps, p 207–221. *In* Yu EW, Zhang Q, Brown MH (ed), Microbial efflux pumps: current research. Caister Academic Press, Inc, Poole, United Kingdom.
3. Warner DM, Folster JP, Shafer WM, Jerse AE. 2007. Regulation of the MtrC-MtrD-MtrE efflux pump system modulates the *in vivo* fitness of *Neisseria gonorrhoeae*. J Infect Dis 196:1804–1812. https://doi.org/10.1086/522964.
4. Grad YH, Harris SR, Kirkcaldy RD, Green AG, Marks DS, Bentley SD, Trees D, Lipsitch M. 2016. Genomic epidemiology of gonococcal resistance to extended-spectrum cephalosporins, macrolides, and fluoroquinolones in the United States, 2000–2013. J Infect Dis 214:1579–1587. https://doi.org/10.1093/infdis/jiw420.
5. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
6. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
7. Chikhi R, Medevedev P. 2014. Informed and automated k-mer size selection for genome assembly. Bioinformatics 30:31–37. https://doi.org/10.1093/bioinformatics/btt310.
8. Zalucki Y, Dhulipala V, Shafer WM. 2012. Dueling regulatory properties of a transcriptional activator (MtrA) and repressor (MtrR) that control efflux pump gene expression in *Neisseria gonorrhoeae*. mBio 3:e00446-12. https://doi.org/10.1128/mBio.00446-12.