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Eshaw Vidyaprakash, Centers for Disease Control and Prevention
A. Jeanine Abrams, Centers for Disease Control and Prevention
William Shafer, Emory University
David L. Trees, Centers for Disease Control and Prevention

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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. Treesa

Division of STD Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, USAa; Department of Microbiology and Immunology and the Emory Antibiotic Resistance Center, Emory University School of Medicine, Atlanta, Georgia, USAb; The Laboratories of Bacterial Pathogenesis, Medical Research Service, VA Medical Center (Atlanta), Decatur, Georgia, USAc

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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/XylS-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 N₅₀ values) were conducted using the SPAdes assembler (5) and the
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.

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We have no competing interests to declare.

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