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BRIEF REPORT

A Genomic Perspective on the Near-term Impact of Doxycycline Post-exposure Prophylaxis on *Neisseria gonorrhoeae* Antimicrobial Resistance

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Pre-existing tetracycline resistance in *Neisseria gonorrhoeae* limits the effectiveness of post-exposure prophylaxis (PEP) with doxycycline against gonorrhea, and selection for tetracycline resistance may influence prevalence of multidrug resistant strains. Using genomic and antimicrobial susceptibility data from *N. gonorrhoeae*, we assessed the near-term impact of doxycycline PEP on *N. gonorrhoeae* resistance.

Pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) with doxycycline has been shown to decrease rates of bacterial sexually transmitted infections (STIs) in clinical trials [1–4], and doxycycline PEP is already used by some men who have sex with men (MSM) [5] and is now recommended by several public health departments in California, including in San Francisco, Santa Clara County, and Alameda County [6–8]. Official guidelines from the US Centers for Disease Control and Prevention (CDC) and other public health institutions in the United States are pending. However, the British Association for Sexual Health and HIV and Public Health England do not currently recommend doxycycline PEP due to the risk of antimicrobial resistance in *N. gonorrhoeae* and other bacterial pathogens [9].

Pre-existing tetracycline resistance in the *N. gonorrhoeae* population resulted in lower doxycycline PEP effectiveness against gonorrhea than against syphilis and chlamydia [2] and reflects tetracycline and multidrug resistant strains of *N. gonorrhoeae* that may be selected for by doxycycline PEP, including strains resistant to current first line treatment. Given this concern, we evaluated the potential near-term impacts of doxycycline PEP on antimicrobial resistance in *N. gonorrhoeae* using whole genome sequencing (WGS) data and minimum inhibitory concentrations (MICs) from a global collection of 5644 *N. gonorrhoeae* isolates (Supplementary Tables 1 and 2), including 1041 isolates from 2018 collected and sequenced by CDC's Gonococcal Isolate Surveillance Program (GISP) [10].

Interpretative breakpoints for *N. gonorrhoeae* susceptibility and resistance to doxycycline have not been defined; however, treatment failures have been observed when isolates have doxycycline MICs ≥ 1 μg/mL [11], and doxycycline MICs correlate with tetracycline MICs [12]. Tetracycline resistance can be mediated by plasmid-encoded *tetM*, which confers high-level resistance, and chromosomally encoded mutations in *rpsJ*, *porB*, and the *mtr* operon [13]. We excluded isolates that had tetracycline MICs that most likely represented reporting errors (Supplementary Text).

We found that co-resistance to other antimicrobials was most common in isolates with chromosomally encoded tetracycline resistance (Figure 1). Ceftriaxone, azithromycin, and ciprofloxacin MICs were significantly higher in isolates with tetracycline MICs of 2–8 μg/mL compared to isolates not resistant to tetracycline and to isolates with high-level tetracycline resistance ($P < .0001$, Mann-Whitney test). Ceftriaxone reduced susceptibility (MIC ≥ 0.125 μg/mL) was rare in GISP isolates (n = 2), but the global data suggested that reduced susceptibility is most often acquired by strains with chromosomally mediated tetracycline resistance, including internationally transmitting strains encoding the *penA* 60 allele, which confers ceftriaxone reduced susceptibility [14, 15]. Azithromycin resistance appeared in 23.5% (56/238) of GISP isolates resistant to tetracycline; all that were tetracycline and azithromycin co-resistant had chromosomally encoded tetracycline resistance. Ciprofloxacin resistance appeared in 33.6% (80/238) of GISP isolates resistant to tetracycline; however, only 12.9% (13/101) of isolates with *tetM* were also ciprofloxacin resistant. Recent work analyzing the presence of resistance-associated alleles in *N. gonorrhoeae* genomes sequenced from EuroGASP showed that lineages encoding *tetM* had fewer additional resistance alleles than lineages encoding tetracycline-resistance associated alleles of *rpsJ* [16], suggesting that we may expect a similar impact of doxycycline PEP on antimicrobial resistance in the United States and Europe.

Doxycycline PEP has primarily been studied in populations including MSM and transgender persons who have sex with men; preliminary results from a clinical trial in cisgender women have been reported, but the final results are pending [17]. The distribution of isolates classified as susceptible (MIC ≤ 0.25 μg/mL), intermediate (0.25 < MIC < 2 μg/mL), resistant (2 ≤ MIC ≤ 8 μg/mL), or high-level resistant (MIC > 8 μg/mL)

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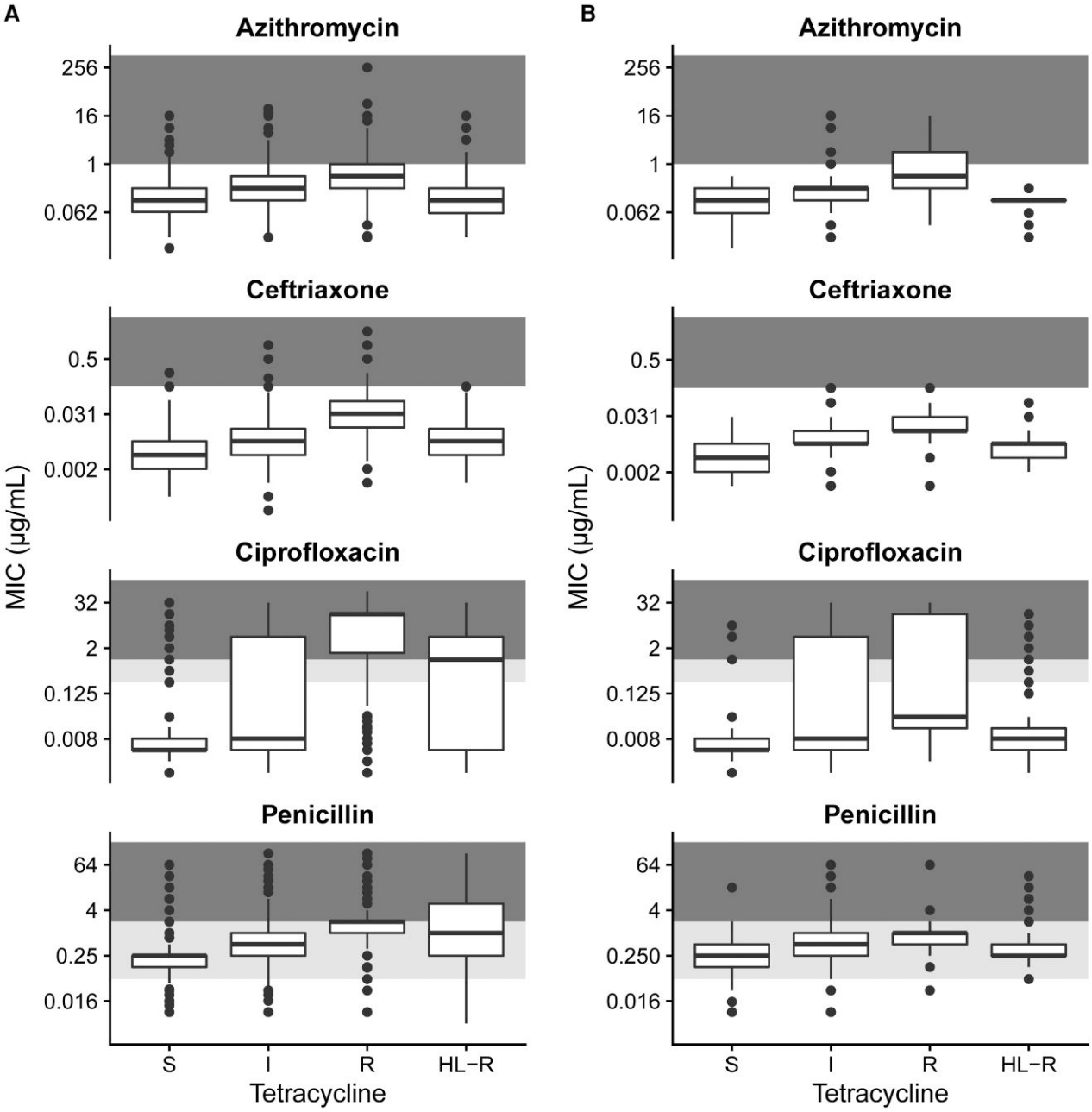

Figure 1. Co-resistance with other antimicrobials is highest among isolates with chromosomally encoded resistance to tetracyclines. Isolates were classified as susceptible (MIC ≤ 0.25 µg/mL), intermediate (0.25 < MIC < 2 µg/mL), resistant (2 ≤ MIC ≤ 8 µg/mL), or high-level resistant (MIC > 8 µg/mL) in 5644 global *Neisseria gonorrhoeae* isolates (*A*) and 1041 isolates collected in the United States in 2018 (*B*). Background shading corresponds to susceptible (white), intermediate (light gray), and resistant/non-susceptible (dark gray) MICs for each antimicrobial. Abbreviation: MIC, minimum inhibitory concentration.

significantly differed between MSM and men who have sex with women (MSW) (Supplementary Table 3, $P < .0001$, χ^2 test). Although the proportion of isolates with tetracycline resistance was similar between MSM (26.8%, 91/340) and MSW (21.3%, 125/587), tetracycline susceptibility was more common among MSW (23.0%, 135/587) compared to MSM (10.3% 35/340). Among MSM, the majority of isolates (62.9%, 214/340) had intermediate tetracycline MICs. In addition to selection for resistant lineages in MSM populations, this large population of *N. gonorrhoeae* with intermediate MICs may represent a reservoir for rapid evolution of resistance. However, the evolutionary pathways and barriers to doxycycline resistance and their variation by genomic background in *N. gonorrhoeae* are unknown.

The composition of *N. gonorrhoeae* isolates was not homogeneous across the United States, and the percentage of

tetracycline resistant isolates collected across Health and Human Services (HHS) regions ranged from 17.4% to 52.0% (Supplementary Figure 1). Mechanisms of resistance also varied, and high-level resistance was not evenly distributed across geographic regions, with the lowest proportion of isolates with high-level resistance in HHS regions from the east coast and southeastern United States (1.7%–4.4%). This indicates that doxycycline PEP will likely have regionally varying effectiveness and impact on circulating *N. gonorrhoeae*.

In our study, the genomic data representing the *N. gonorrhoeae* population in the United States were from isolates collected in 2018, and recent changes in treatment guidelines [18] and introductions of resistant lineages, such as isolates encoding *penA* 60 [19], may have shifted the landscape of antimicrobial resistance in the United States. The number of genomes meeting our quality thresholds from the southeastern United States was low (Supplementary Figure 1), so these data may not accurately capture lineages transmitting in this region. We emphasize that the long-term impacts of doxycycline PEP on the *N. gonorrhoeae* population in the United States are unknown, as circulating lineages may acquire new resistance alleles [20–22]. Additionally, epidemic spread of *N. gonorrhoeae* with high-level tetracycline resistance has been described in other geographic regions, and introduction of these lineages to the United States is possible [23, 24]. For example, a recent study found that strains encoding *tetM* are highly prevalent among women in Kenya [25], and phylogenetic analysis of our global dataset identified several lineages with high-level tetracycline resistance not represented among GISP isolates (Supplementary Figure 2).

The near-term impact of doxycycline PEP on *N. gonorrhoeae* antimicrobial resistance and circulating lineages will be influenced by the strength of selection for plasmid-encoded tetracycline resistance compared to chromosomally-encoded tetracycline resistance. If doxycycline PEP use selects for all tetracycline resistant lineages, there is potential for increased resistance to other antimicrobials as well. However, if doxycycline PEP use primarily selects for lineages with *tetM*-mediated resistance, we may observe a temporary decline in resistance to other antimicrobials due to relatively less co-resistance in these lineages. Timely genomic surveillance, with a focus on doxycycline PEP users, is needed to distinguish between these outcomes and to detect the emergence of novel combinations of resistance-associated alleles.

METHODS

Data Set

Whole genome sequencing data and minimum inhibitory concentrations were collected from previously published *N. gonorrhoeae* genomic studies (Supplementary Table 1). Isolates were included if the tetracycline MIC was reported with sufficient precision to categorize isolates as susceptible, intermediate, resistant, or high-level resistant.

Genome Assembly

Whole genomes sequencing data were assembled as previously described [26]. Briefly, we used SPAdes v 3.12.0 [27] for de novo assembly; the –careful flag was used to correct assemblies, and contigs were removed if coverage was <10× or contig length was <500 nucleotides. Reads were additionally mapped to the NCCP11945 reference genome (NC_011035.1) using BWA-MEM v 0.7.17 [28], and variants were called using Pilon v 1.23 [29] with a minimum mapping quality of 20 and 10× minimum coverage.

Quality Control of Genomic Data

We included isolates with genomes meeting the following quality control filters: the number of contigs was <500, the assembly length and number of annotated genes was expected for *N. gonorrhoeae,* coverage was >30×, at least 80% of reads mapped to the *N. gonorrhoeae* reference genome, and fewer than 12% of sites in the *N. gonorrhoeae* reference genome were unable to be confidently called using our assembly pipeline. Quality control metrics for included genomes is summarized in Supplementary Table 2. Additionally, we required that the contig encoding *tetM* was >10 000 nucleotides in length and at least 15× coverage.

Phylogenetic Analysis

We used pseudogenomes derived from reference mapping and variant calls for phylogenetic analysis; we required that 90% of reads supported an allele to include the position in an isolate's pseudogenome. We used Gubbins v 2.4.1 [30] to identify recombinant regions and reconstruct the phylogeny.

Statistical Analysis

All statistical analysis was performed in R v 4.1.3 [31]. Significance of differences between MICs to ceftriaxone, azithromycin, ciprofloxacin, and penicillin was assessed using a Mann-Whitney test. Significance of differences of susceptible, intermediate, resistant, and high-level resistant isolates among demographic groups was assessed using a χ^2 test.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. T. D. M. performed data curation and analysis. Y. H. G. supervised and managed the study. T. D. M. and Y. H. G. wrote the manuscript, had full access to the data reported, and were responsible for the decision to submit the manuscript for publication.

Data availability. Data and code are available at https://github.com/gradlab/doxyPEP_genomics.

Disclaimer. The findings, conclusions, and views expressed are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

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Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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