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Investigating the genetic modulators of observed vancomycin susceptibility in *Neisseria gonorrhoeae* isolates

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### Author(s)

Rebecca M. McSweeney, n/a

Research Assistant I Lab

Grad laboratory, Harvard T.H. Chan School of Public Health Department of Immunology and Infectious Diseases

Daniel Rubin, PhD

MD/PhD Student

Harvard Medical School

Samantha Palace, PhD

Research Associate

Harvard T.H. Chan School of Public Health

Aditya Bandekar, PhD

Postdoctoral Fellow

Harvard T.H. Chan School of Public Health

Tatum Mortimer, PhD

Postdoctoral Research Fellow

Harvard T.H. Chan School of Public Health

Yonatan Grad, M.D., Ph.D.

Melvin J. and Geraldine L. Glimcher Associate Professor of Immunology and Infectious Diseases

Harvard T.H. Chan School of Public Health

### Background

*Neisseria gonorrhoeae* differs from other Gram-negative bacteria in that a subset of isolates is susceptible to vancomycin, a glycopeptide that binds the stem peptide of peptidoglycan. The true prevalence of vancomycin susceptible *N. gonorrhoeae* is not known, because vancomycin is routinely included in media—for example, at 3 µg/mL in Thayer-Martin—used to culture *N. gonorrhoeae* from patient specimens. Moreover, while variant alleles of the efflux pump *mtr*, the major porin *porB*, and the lytic transglycosylases have been reported to influence *N. gonorrhoeae*'s vancomycin susceptibility, the full set of genetic modulators of vancomycin resistance is not known.

### Aim/Methods

We measured the vancomycin minimum inhibitory concentrations (MICs) of 212 genetically diverse *N.*

gonorrhoeae isolates by agar dilution and ETEST. We then performed a genome-wide association study (GWAS) to identify genetic factors associated with vancomycin MIC. We cultured susceptible strains to investigate their growth on Thayer-Martin media and performed undirected and targeted transformations to identify loci that confer vancomycin resistance.

## Results

We identified fourteen (6.6%) vancomycin-susceptible strains ( $\text{MIC} \leq 3 \mu\text{g/mL}$ ). The *mtr*, *porB*, and lytic transglycosylase alleles were not significantly associated with increased vancomycin resistance by GWAS, and known variants in these loci account for less than 50 percent of variation in vancomycin MIC. Seven of the fourteen vancomycin-susceptible strains grew on Thayer-Martin agar with  $3 \mu\text{g/mL}$  vancomycin after two overnight incubations, but susceptible isolates from patients with disseminated gonococcal infection—in which isolates are often initially cultured from patient blood specimens on non-selective media—did not.

## Conclusions

While *mtr*, *porB*, and lytic transglycosylases explain some variation in vancomycin MIC, other genetic factors contribute to vancomycin susceptibility in *N. gonorrhoeae*. The ability of susceptible strains to grow on vancomycin-containing media varied by infection site, possibly due to previous specimen collection on selective media.