

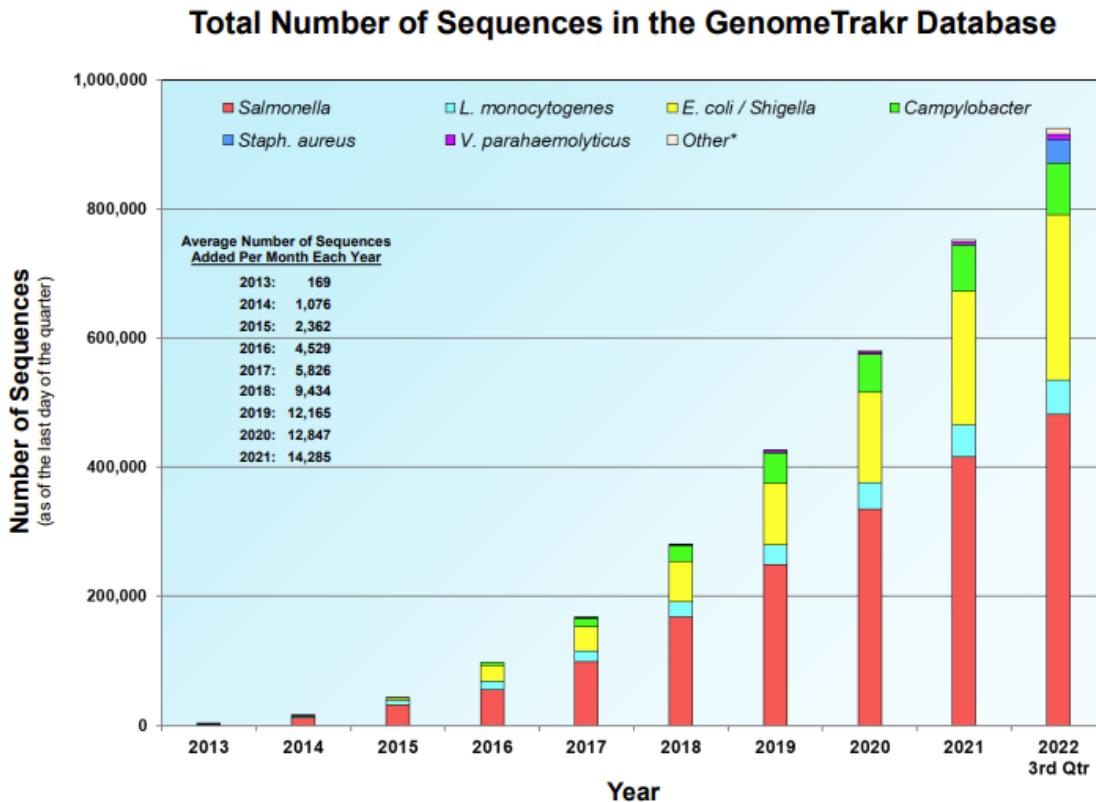
Neisseria gonorrhoeae adaptation to antimicrobials and host-associated niches

Tatum Mortimer

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Harvard T.H. Chan School of Public Health

Pathogen genomes are a rich and growing data source

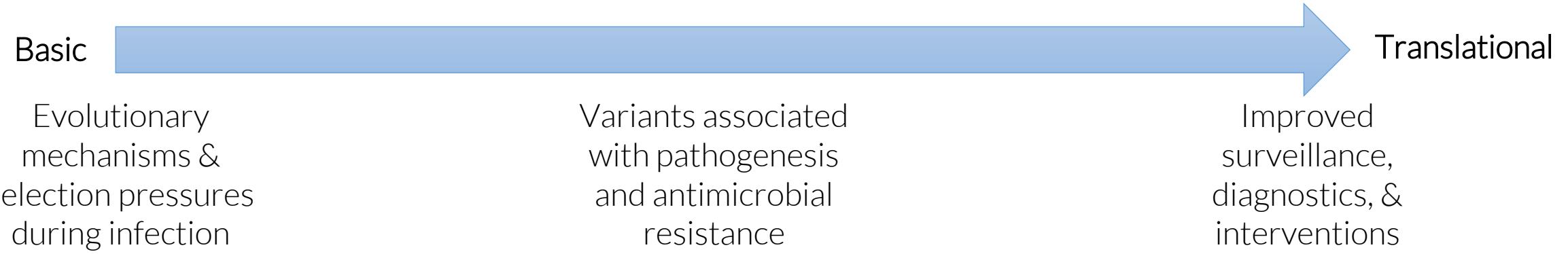


FDA

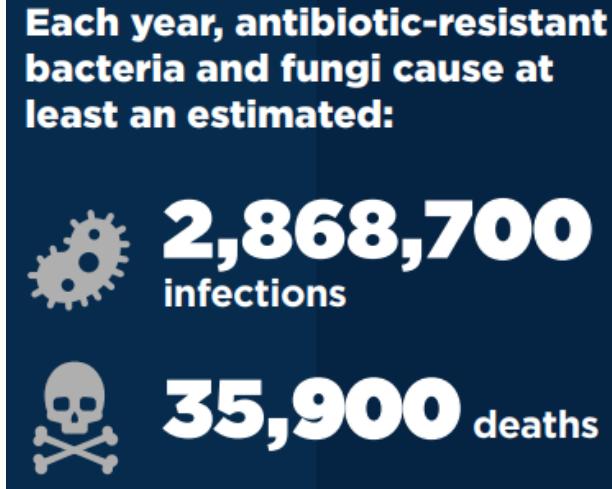
Available Genomic Data (NCBI)

	Total Genomes
<i>Escherichia coli</i>	200,000+
<i>Staphylococcus aureus</i>	97,000+
<i>Klebsiella pneumoniae</i>	63,000+
<i>Neisseria gonorrhoeae</i>	38,000+
<i>Enterococcus faecium</i>	29,000+
<i>Pseudomonas aeruginosa</i>	26,000+

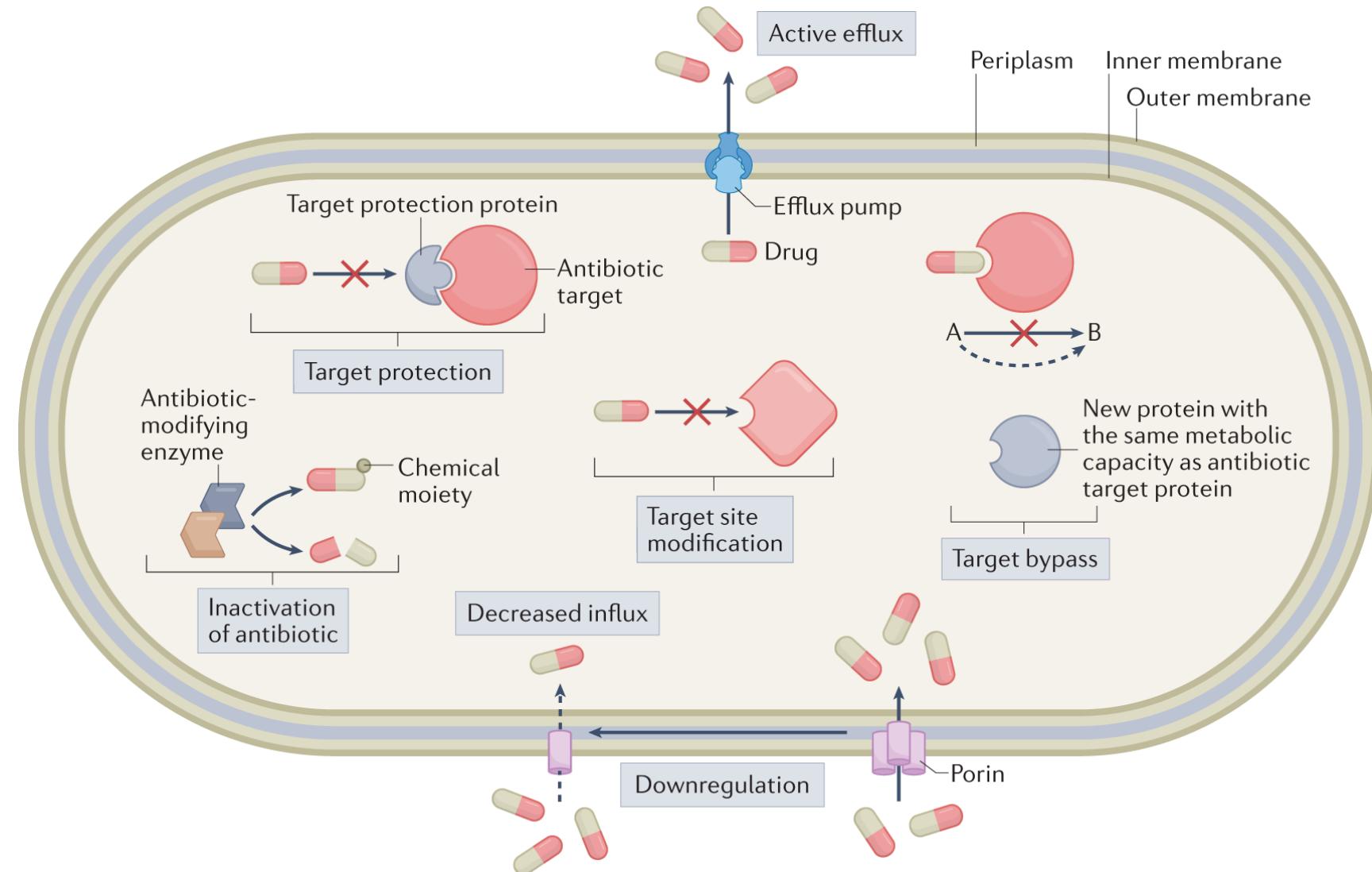
Research Program



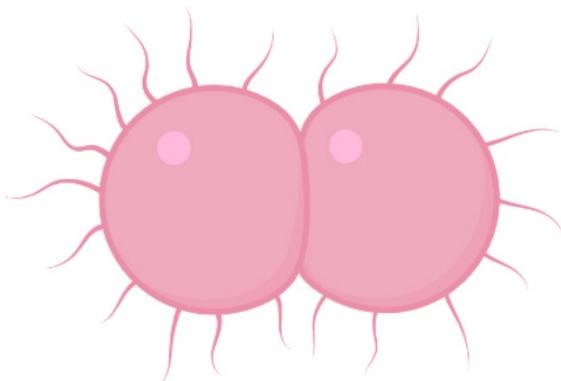
Antimicrobial resistance (AMR) is a public health threat



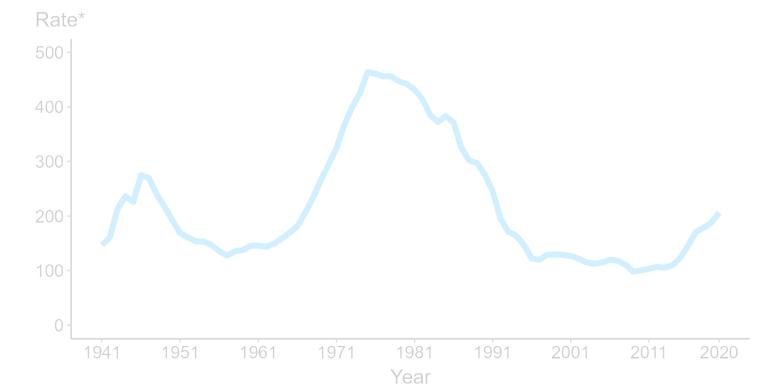
Need to reduce the acquisition and spread of antimicrobial resistance and develop new public health and clinical interventions



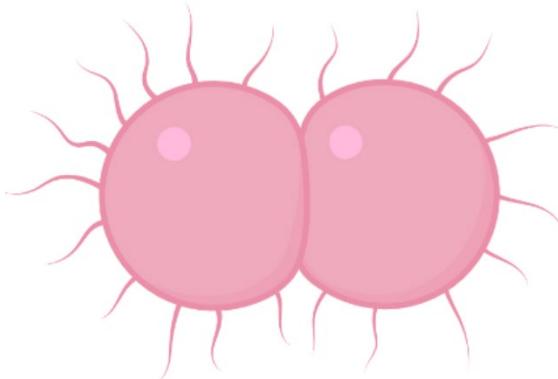
Neisseria gonorrhoeae



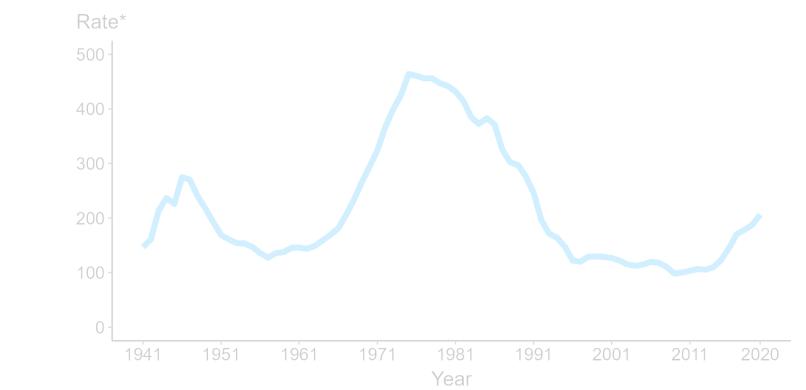
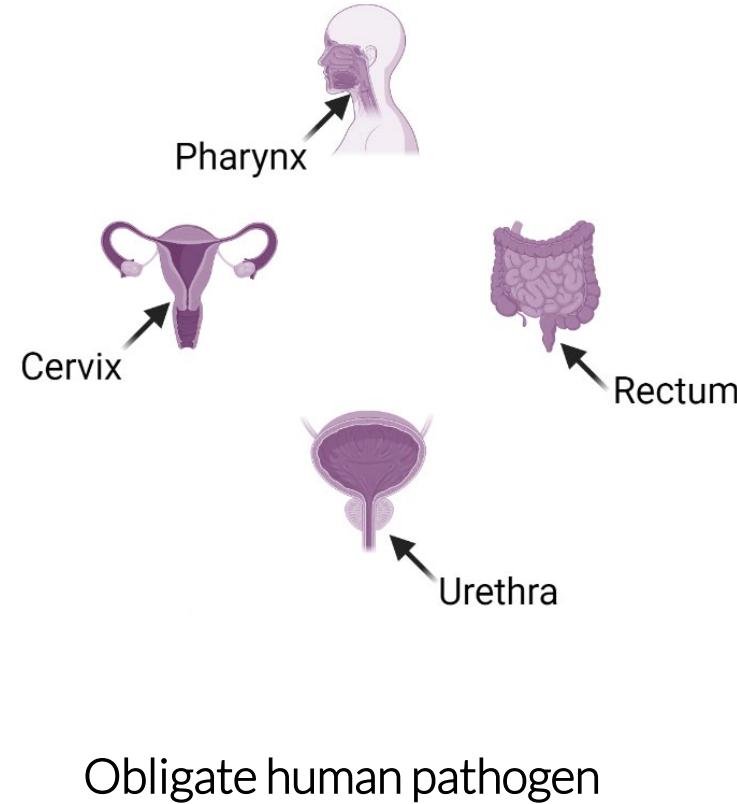
Gram-negative diplococcus



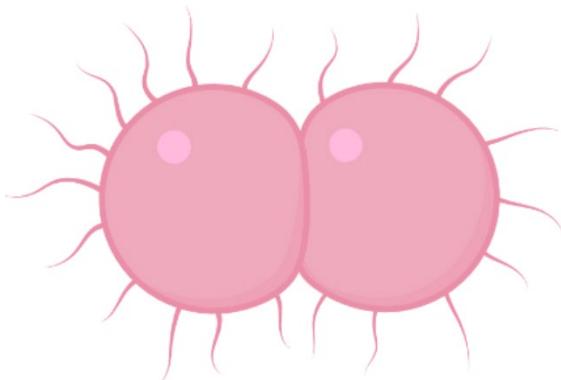
Neisseria gonorrhoeae



Gram-negative diplococcus



Neisseria gonorrhoeae



Gram-negative diplococcus

Conjunctivitis



Pharynx



Cervix



Disseminated Gonococcal Infection



Rectum

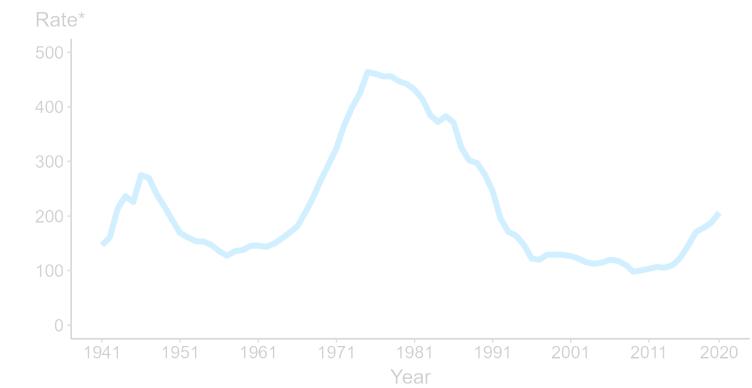


Urethra

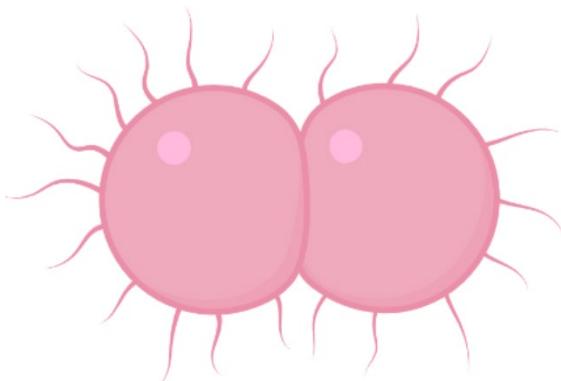
Pelvic Inflammatory Disease



Obligate human pathogen



Neisseria gonorrhoeae



Gram-negative diplococcus

Conjunctivitis

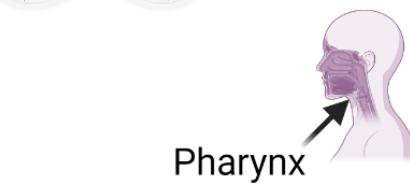


Pelvic
Inflammatory
Disease



Obligate human pathogen

8



Pharynx



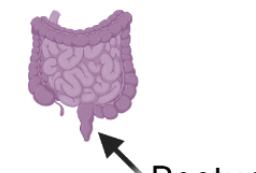
Cervix



Urethra

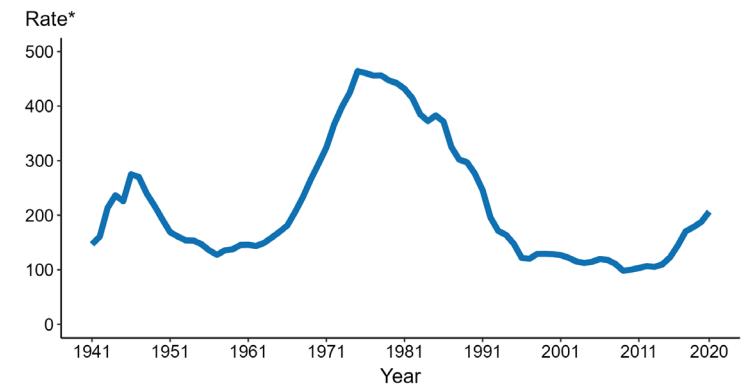


Disseminated
Gonococcal
Infection



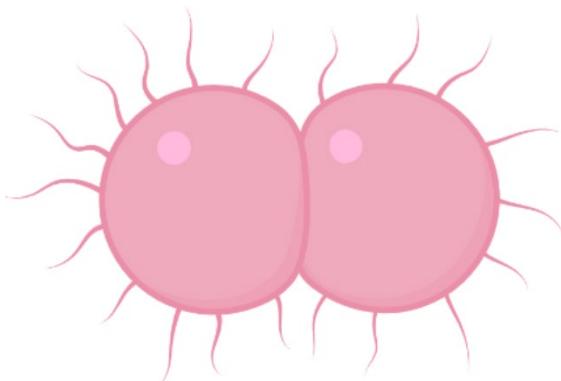
Rectum

Rates of Reported Cases per 100,000, United States



Increasing in incidence

Neisseria gonorrhoeae



Gram-negative diplococcus

Conjunctivitis



Pharynx



Cervix



Urethra

Pelvic Inflammatory Disease

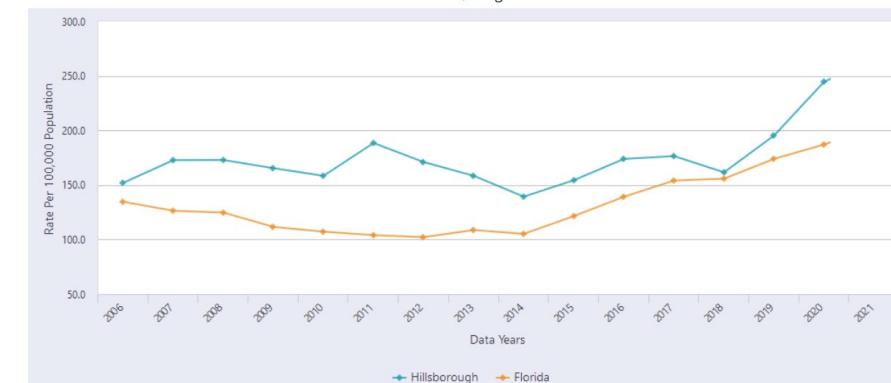


Obligate human pathogen

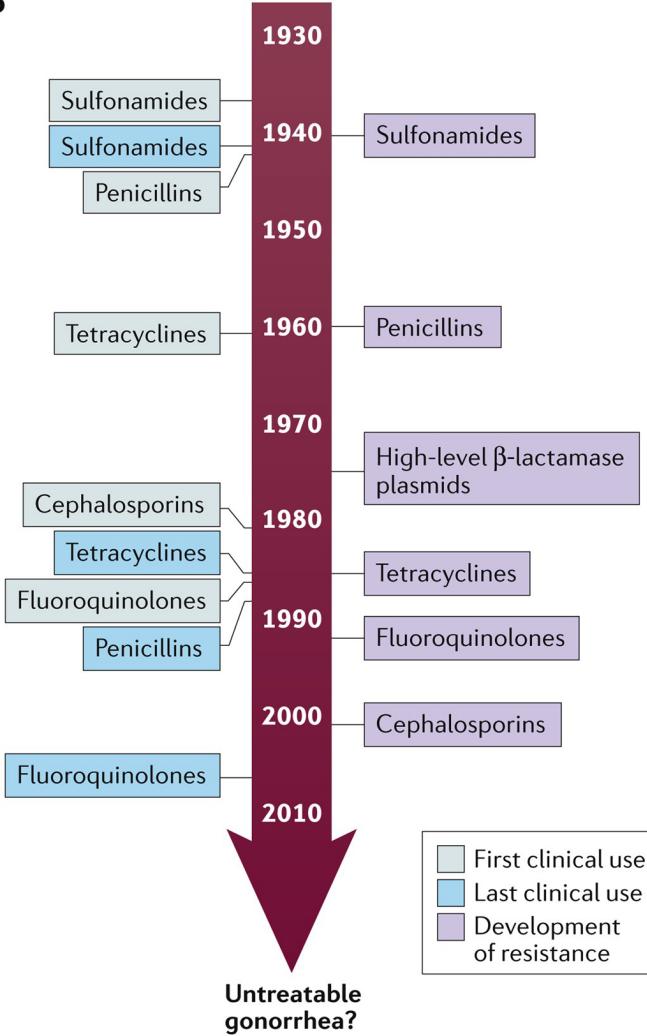
Disseminated Gonococcal Infection



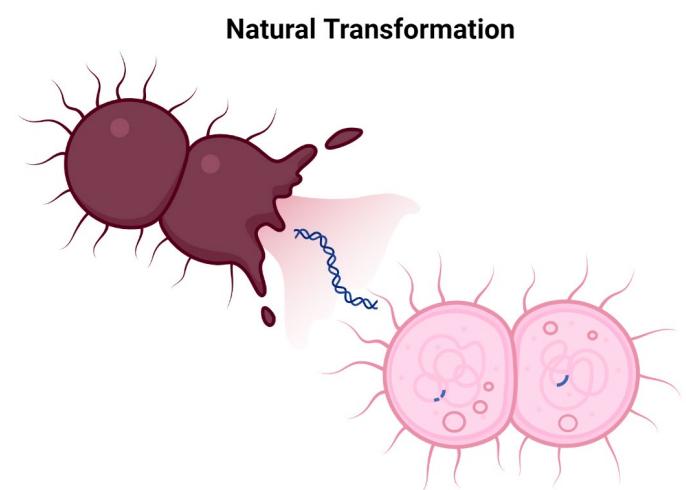
Rates of Reported Cases per 100,000, Florida



Increasing in incidence



Nature Reviews | Microbiology



Recent updates:

Growing azithromycin resistance
 ↓
 Transition to monotherapy with ceftriaxone

Detection of 10 cases of ceftriaxone-resistant *Neisseria gonorrhoeae* in the United Kingdom, December 2021 to June 2022

Michaela Day¹, Rachel Pitt¹, Nisha Mody¹, John Saunders¹, Rupa Rai¹, Achyuta Nori¹, Hannah Church¹, Sarah Mensforth¹, Helen Corkin¹, Jacqueline Jones², Preneshni Naicker³, Wazirzada M Khan¹, Rebecca Thomson Glover¹, Kalani Mortimer¹, Chloe Hylton¹, Elizabeth Moss¹, Thomas Joshua Pasvol¹, Ania Richardson¹, Suzy Sun¹, Neil Woodford¹, Hamish Mohammed¹, Katy Sinka¹, Helen Fifer¹

Outline

- Part 1: Genomic epidemiology of resistant and susceptible *N. gonorrhoeae*
- Part 2: Targets for point of care diagnostics
- Part 3: Increased antibiotic susceptibility associated with adaptation to host environment
- Ongoing Work and Future Directions

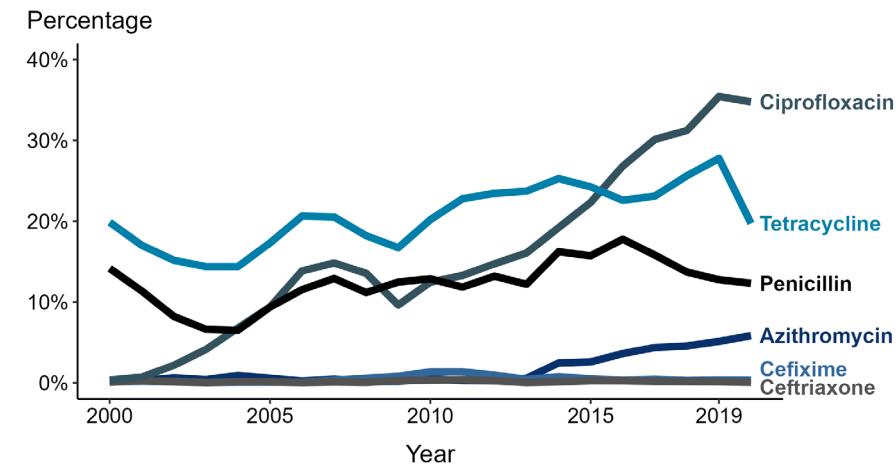
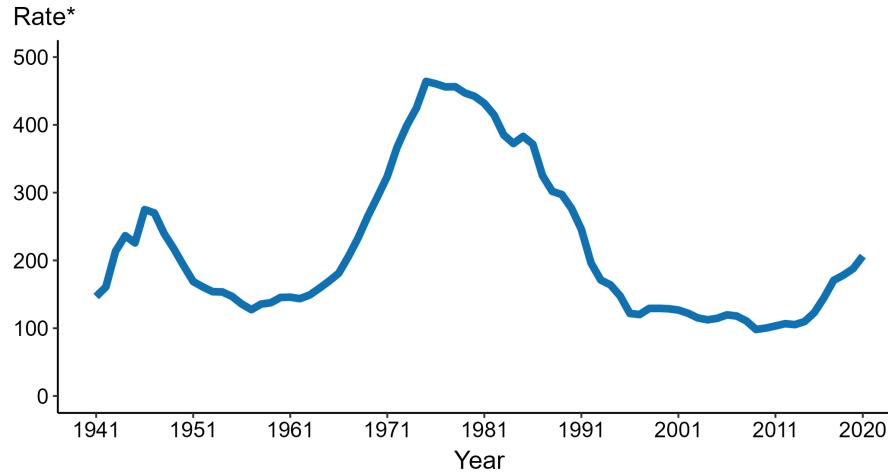
Part 1: Genomic epidemiology of resistant and susceptible *Neisseria gonorrhoeae*

Clinical Infectious Diseases

The Distribution and Spread of Susceptible and Resistant
Neisseria gonorrhoeae Across Demographic Groups in a
Major Metropolitan Center

Tatum D. Mortimer,^{1,○} Preeti Pathela,² Addie Crawley,² Jennifer L. Rakeman,³ Ying Lin,³ Simon R. Harris,⁴ Susan Blank,^{2,5} Julia A. Schillinger,^{2,5,○} and
Yonatan H. Grad,^{1,6,○}

Incidence and AMR are increasing



Increasing in incidence



?

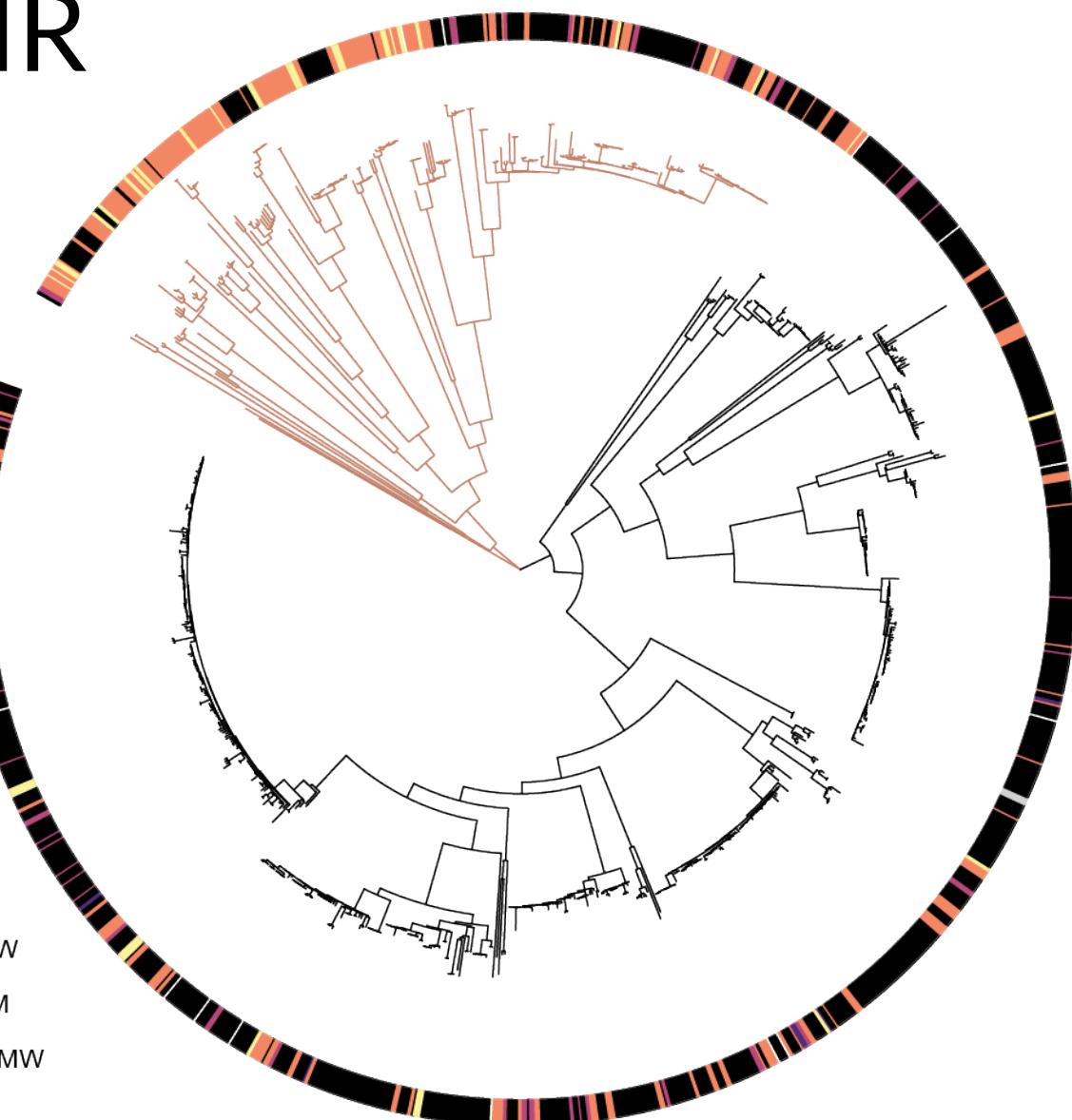
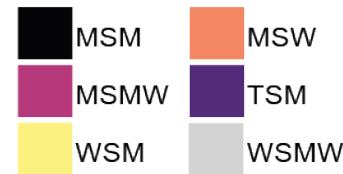
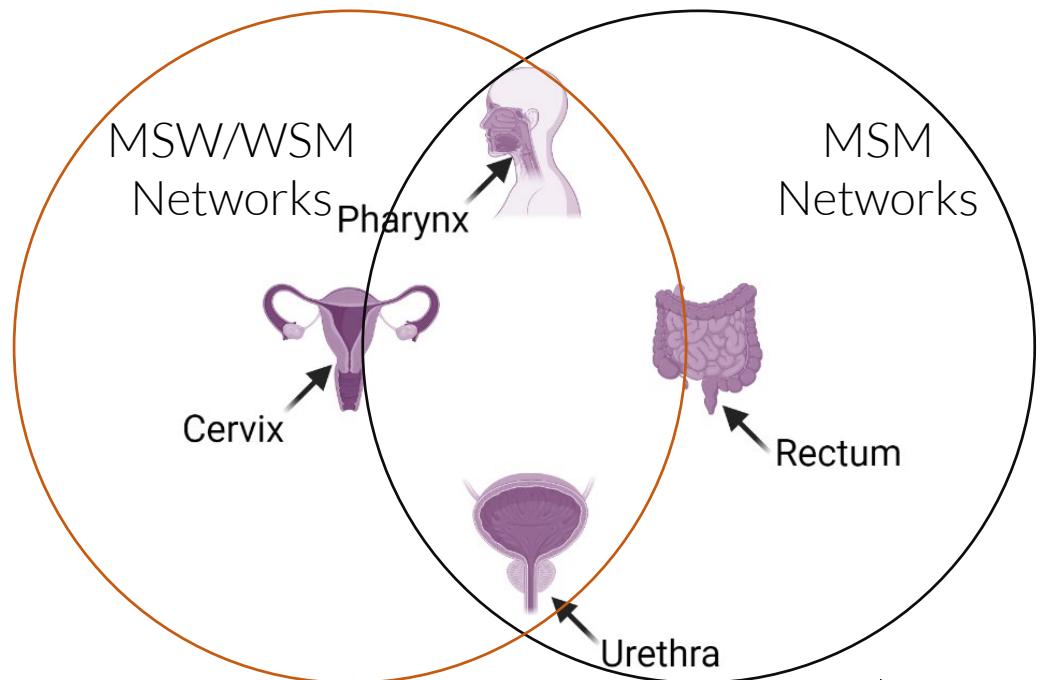
Increasing resistance

Part 1 Goal:

Define transmission networks and investigate interactions between patient demographics and antibiotic resistance

N. gonorrhoeae population is shaped by sexual behavior and AMR

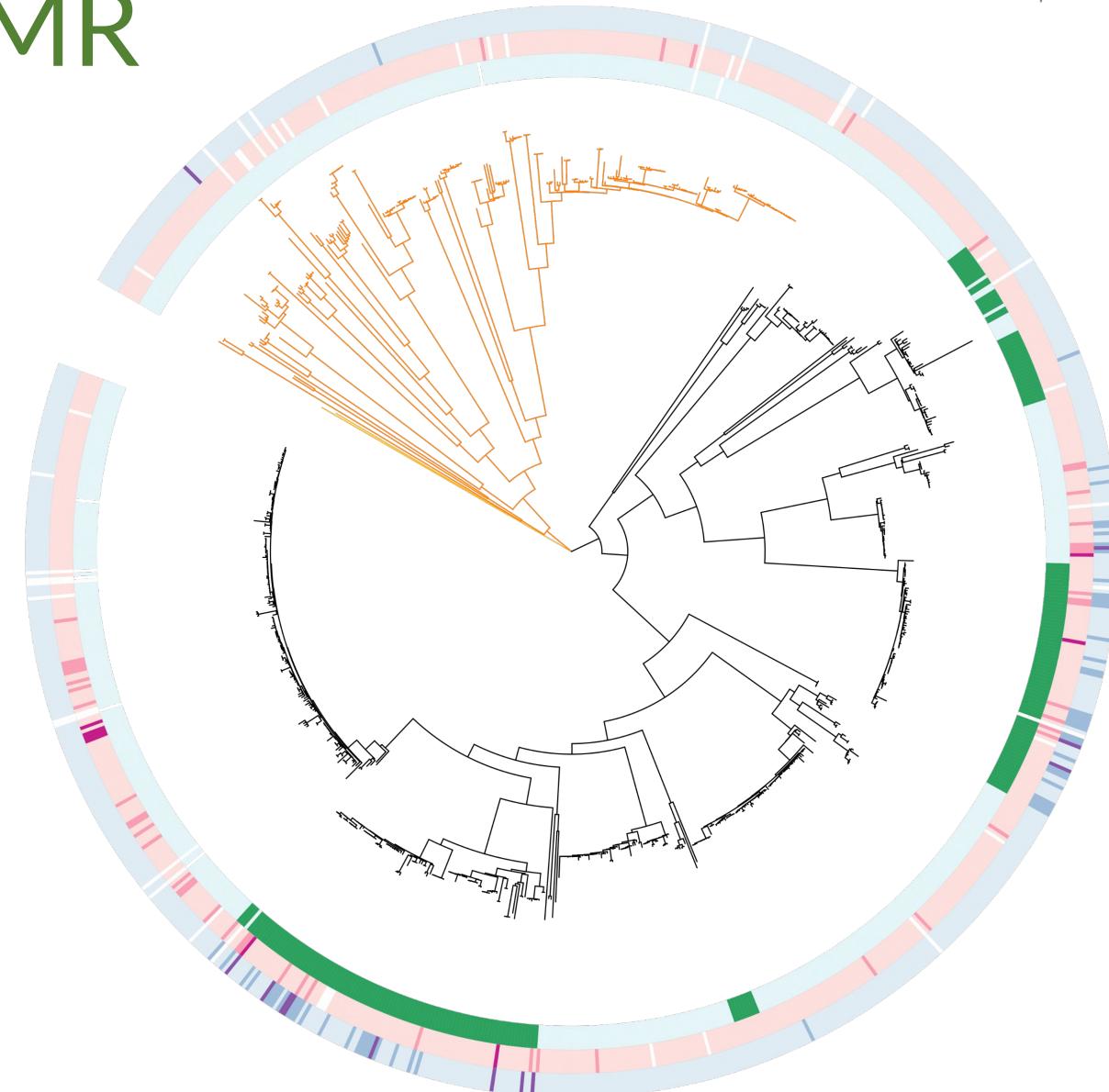
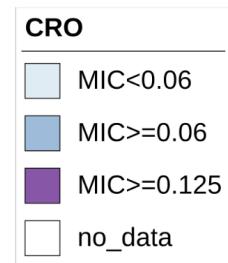
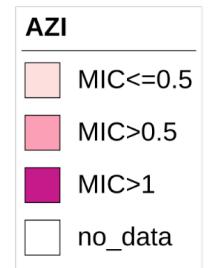
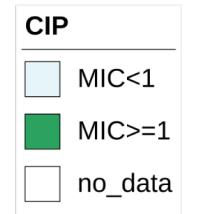
1000 SNPs



N. gonorrhoeae population is shaped by sexual behavior and AMR

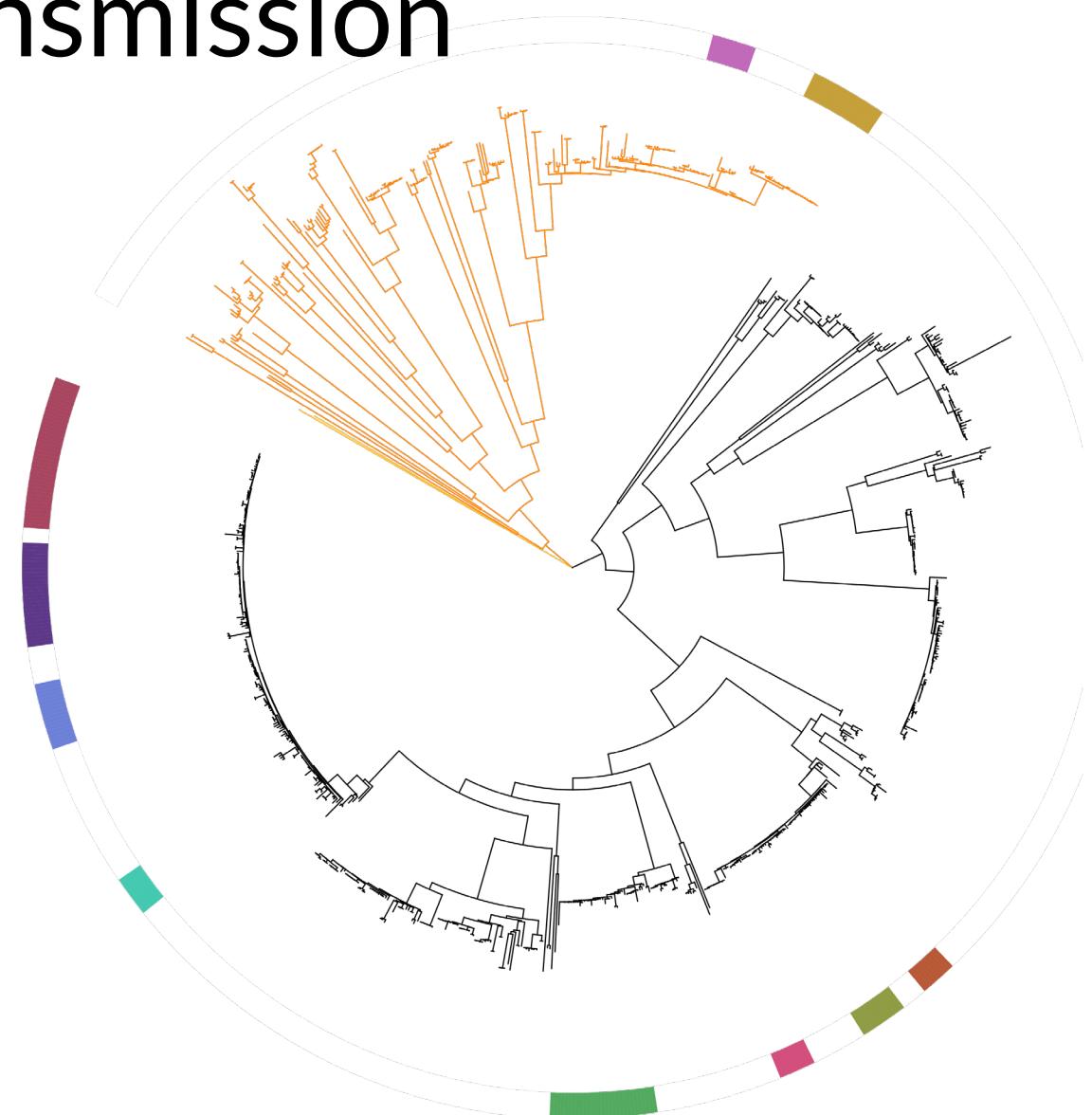
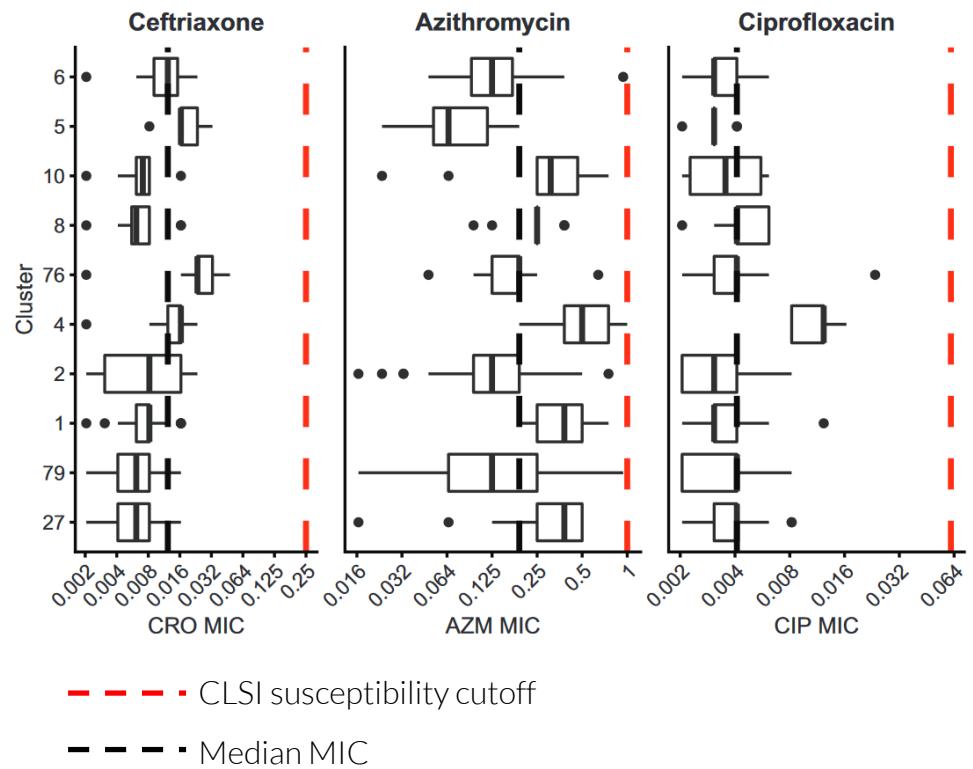
1000 SNPs
—

MIC: Minimum Inhibitory Concentration



Antimicrobial resistance is not the primary driver of gonorrhea transmission

1000 SNPs
—



Part 1: Conclusions

- *N. gonorrhoeae* population is structured by sexual behavior and antimicrobial resistance
- The most successful lineages from the largest transmission clusters are susceptible to current and previous recommended therapy.
 - Antimicrobial resistance is not the primary driver of increased incidence, and new interventions are needed.
 - Suggests that if antimicrobial susceptibility was known, diverse antibiotics could be reintroduced into clinical use.

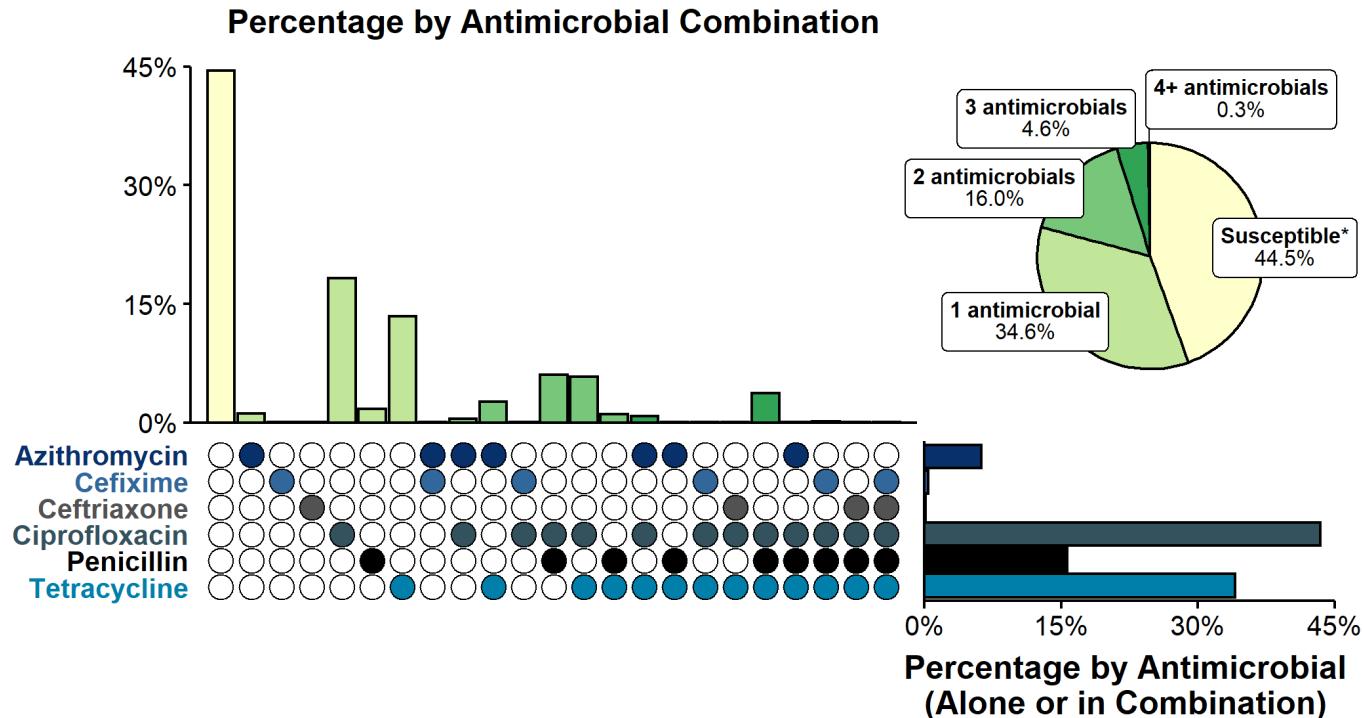
Part 2: Genetic targets for point of care AMR diagnostics

Lancet Microbe

Loci for prediction of penicillin and tetracycline susceptibility in *Neisseria gonorrhoeae*: a genome-wide association study

Tatum D Mortimer*, Jessica J Zhang*, Kevin C Ma, Yonatan H Grad

Many isolates are not resistant

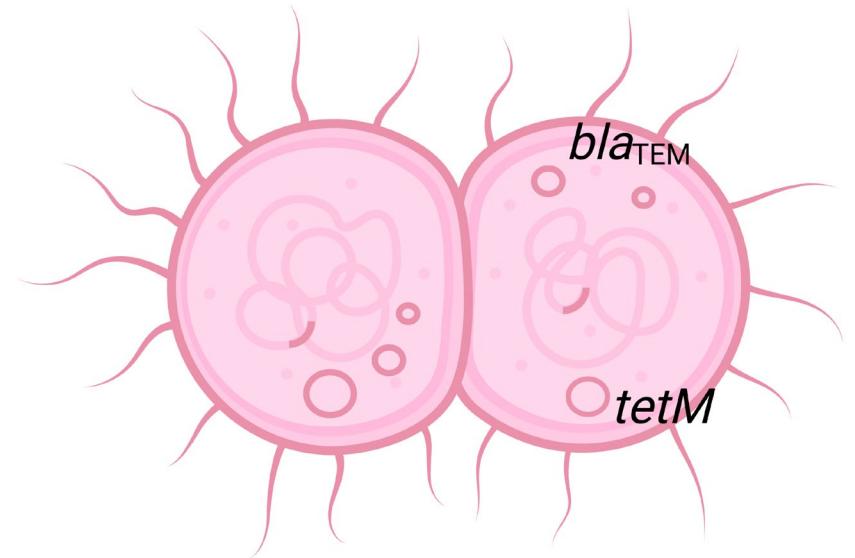


ResistancePlus® GC



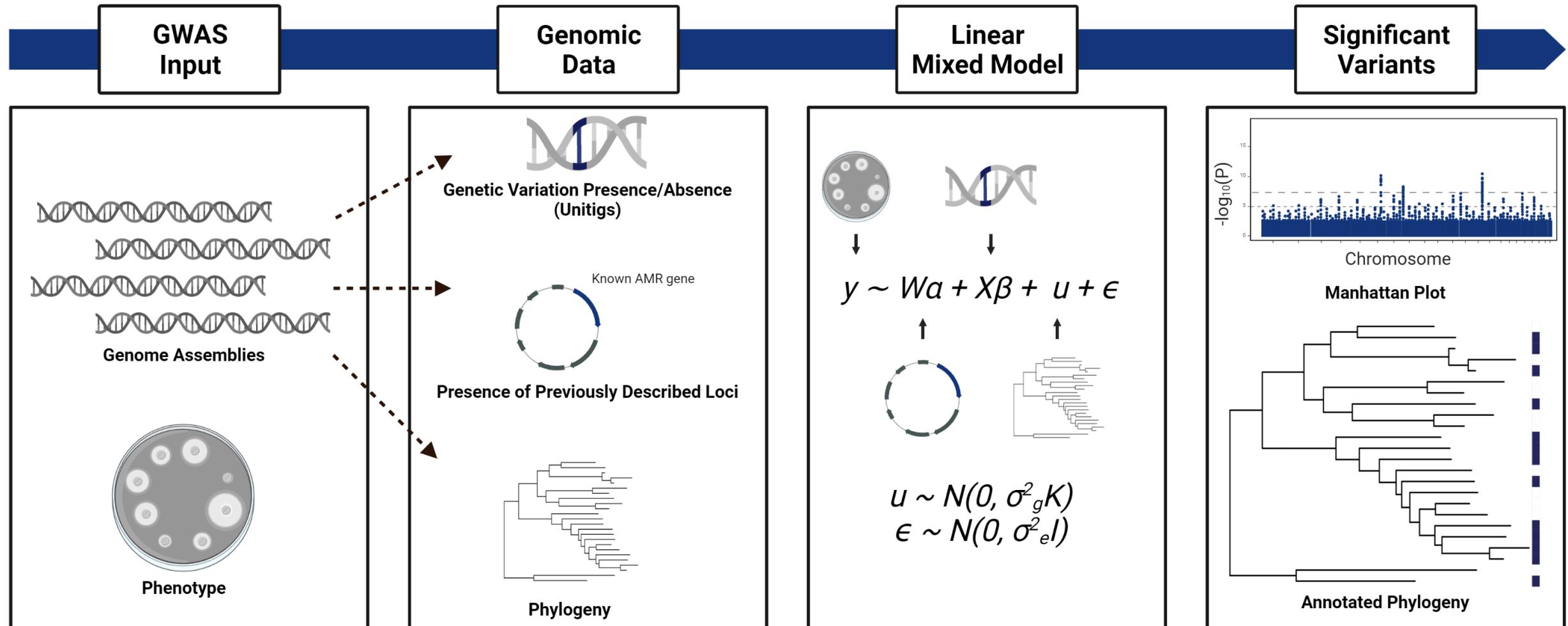
Treating gonorrhea with penicillin and tetracycline

- Penicillin and tetracycline were used for gonorrhea treatment for decades until the emergence of resistance
- Discontinued in the mid-late 1980s
- High level resistance is mediated by plasmid encoded bla_{TEM} and $tetM$
- Chromosomally-encoded resistance is mediated by variants in antibiotic targets, efflux pump upregulation, and reduced antibiotic influx via porin

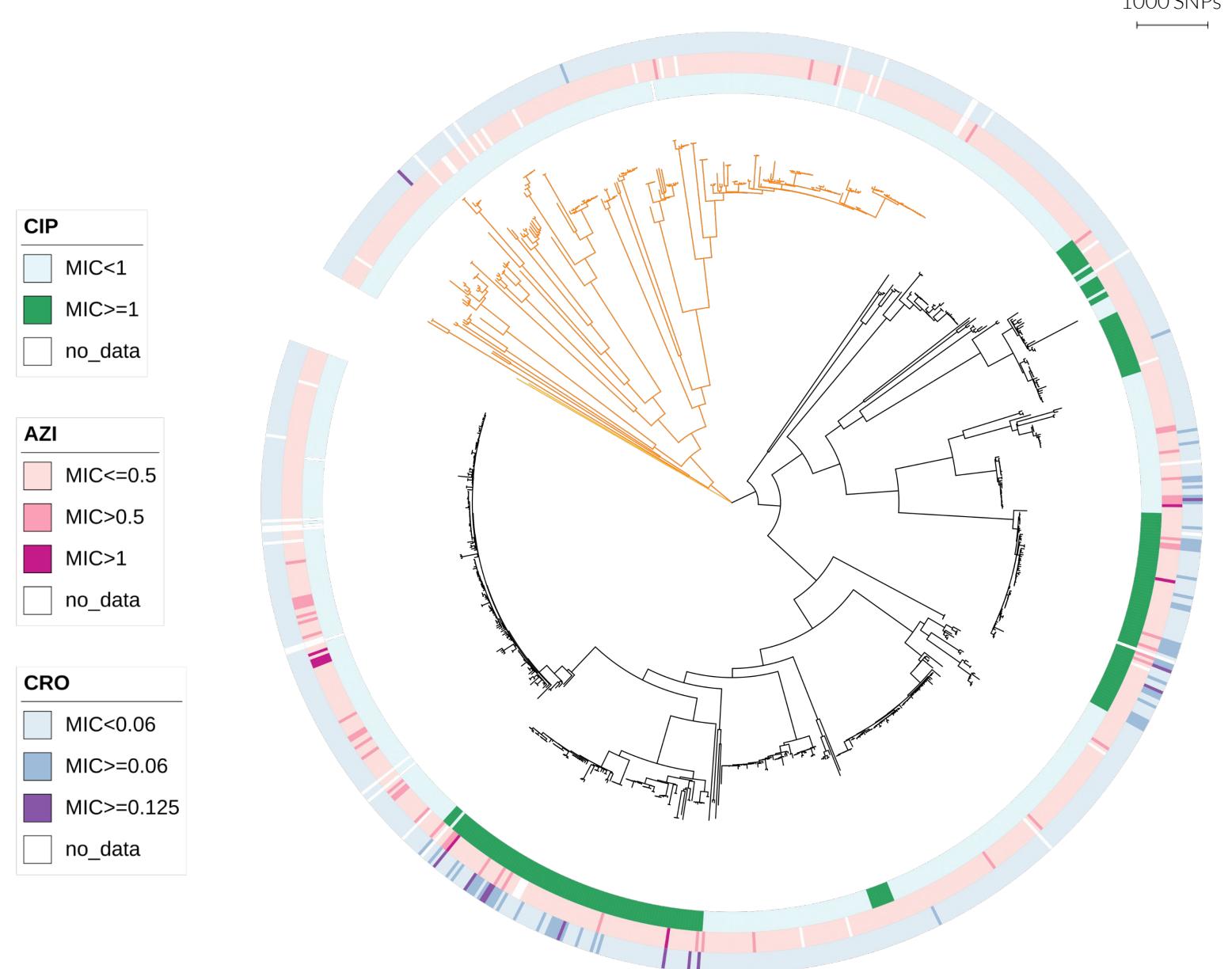


Part 2 Goal:
Identify variants that predict penicillin and
tetracycline susceptibility

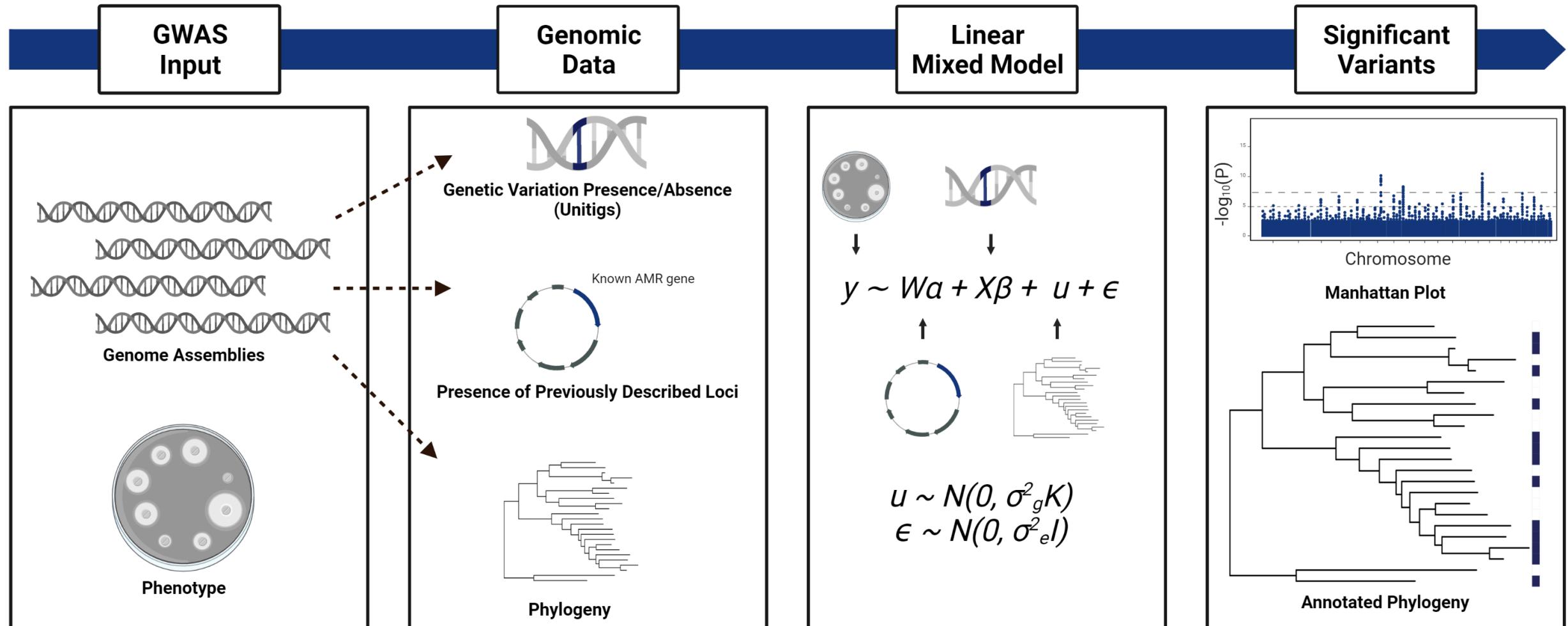
Genome wide association studies



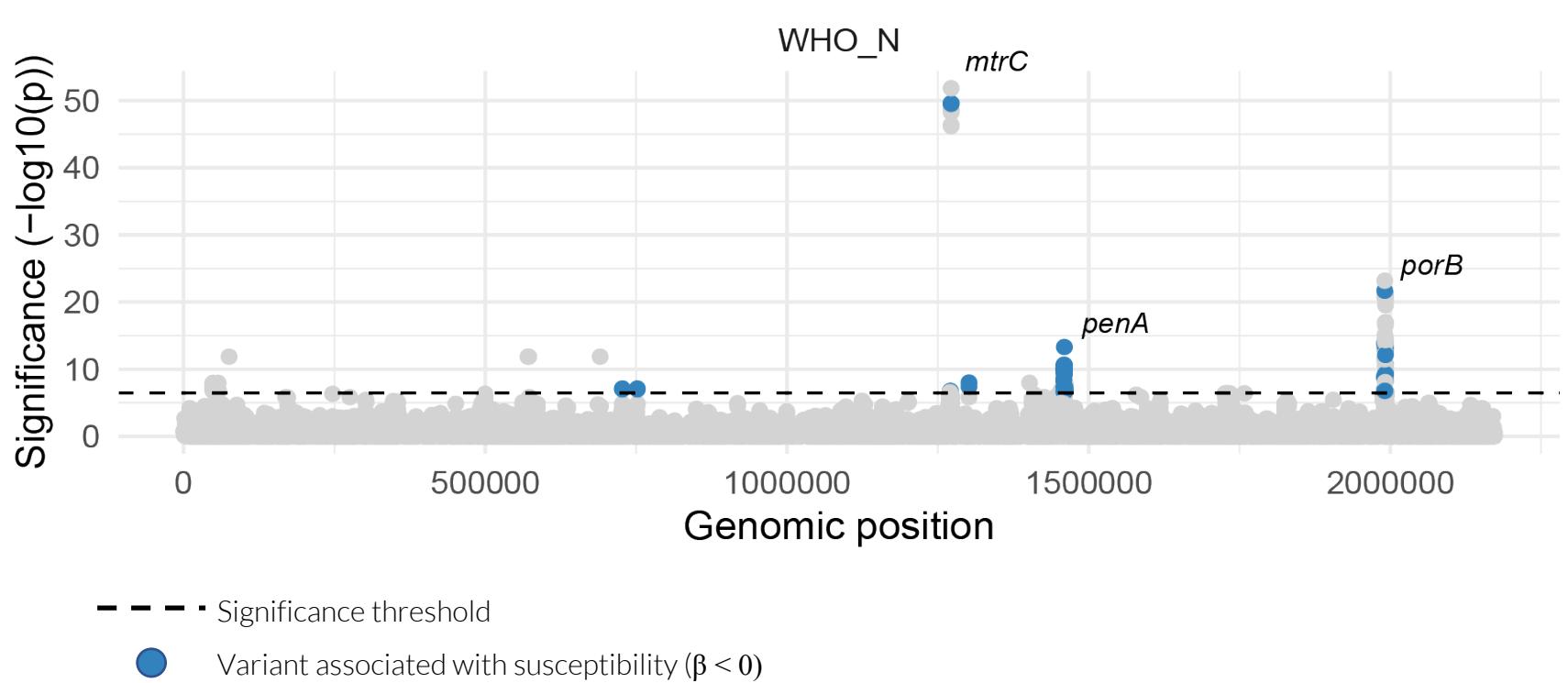
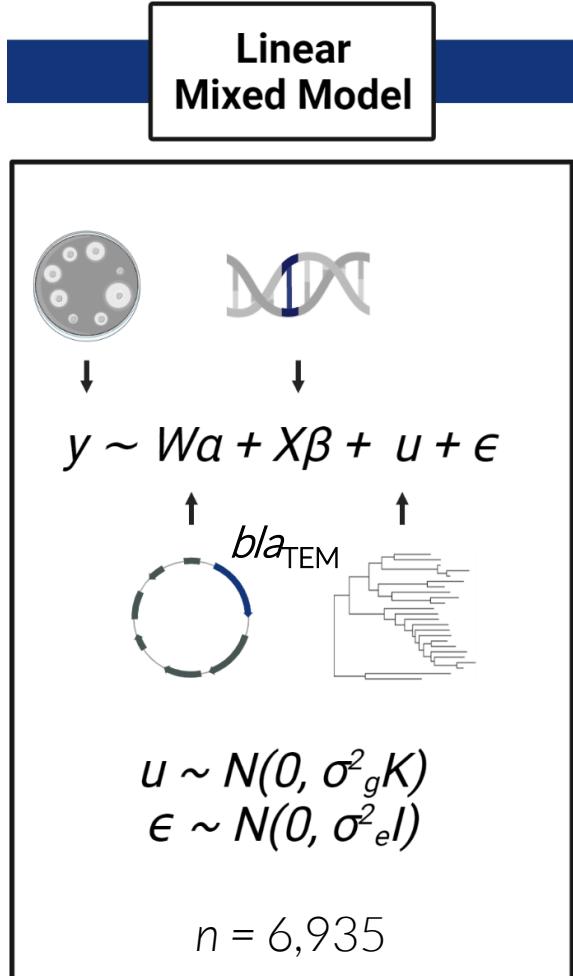
Population structure can lead to GWAS false positives



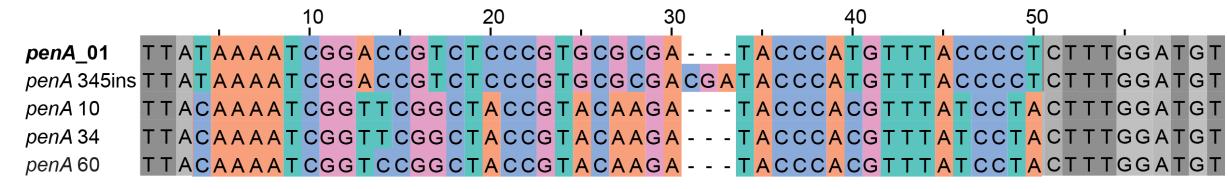
Genome wide association studies



Penicillin conditional GWAS



penA_01 unitig differentiates susceptible allele from two known resistance alleles



$$p = 5.0 \times 10^{-14}, \beta = -2.5$$

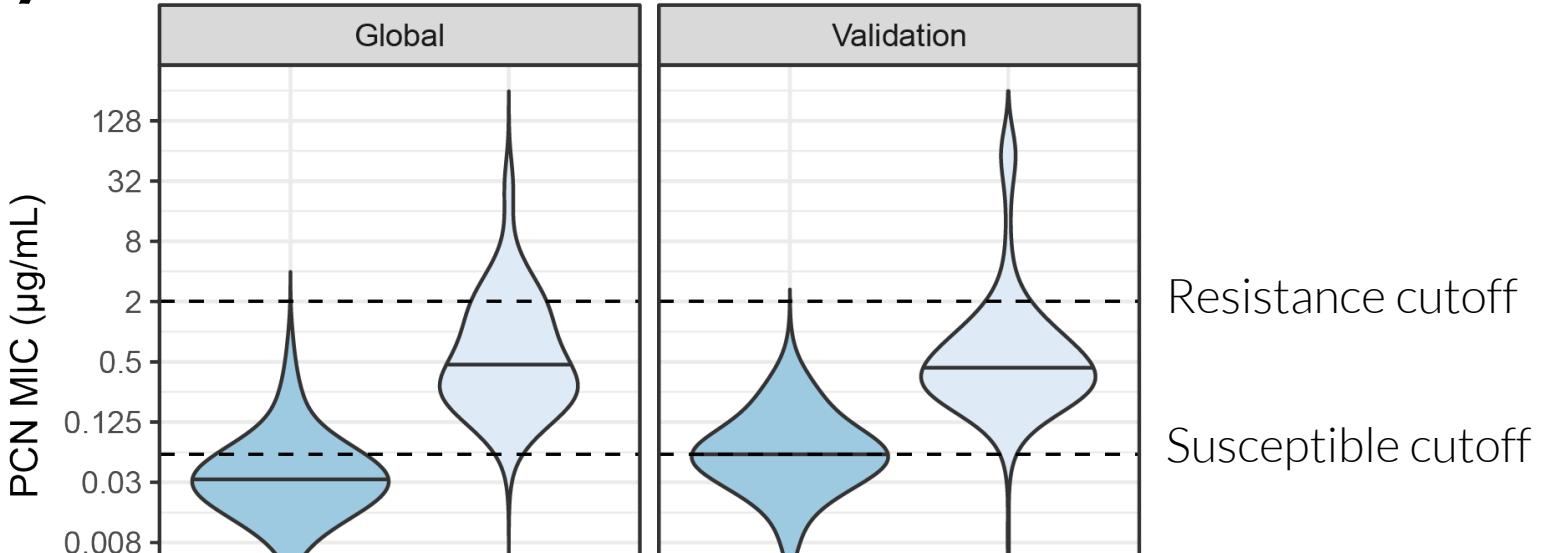
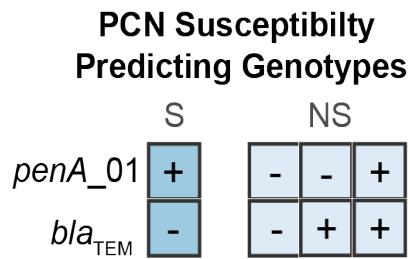
Insertion of an extra amino acid is the main cause of the low affinity of penicillin-binding protein 2 in penicillin-resistant strains of *Neisseria gonorrhoeae*

J. A. Brannigan, I. A. Tirodimos,[†] Q.-Y. Zhang,
C. G. Dowson and B. G. Spratt*

Role of Interspecies Transfer of Chromosomal Genes in the Evolution of Penicillin Resistance in Pathogenic and Commensal *Neisseria* Species

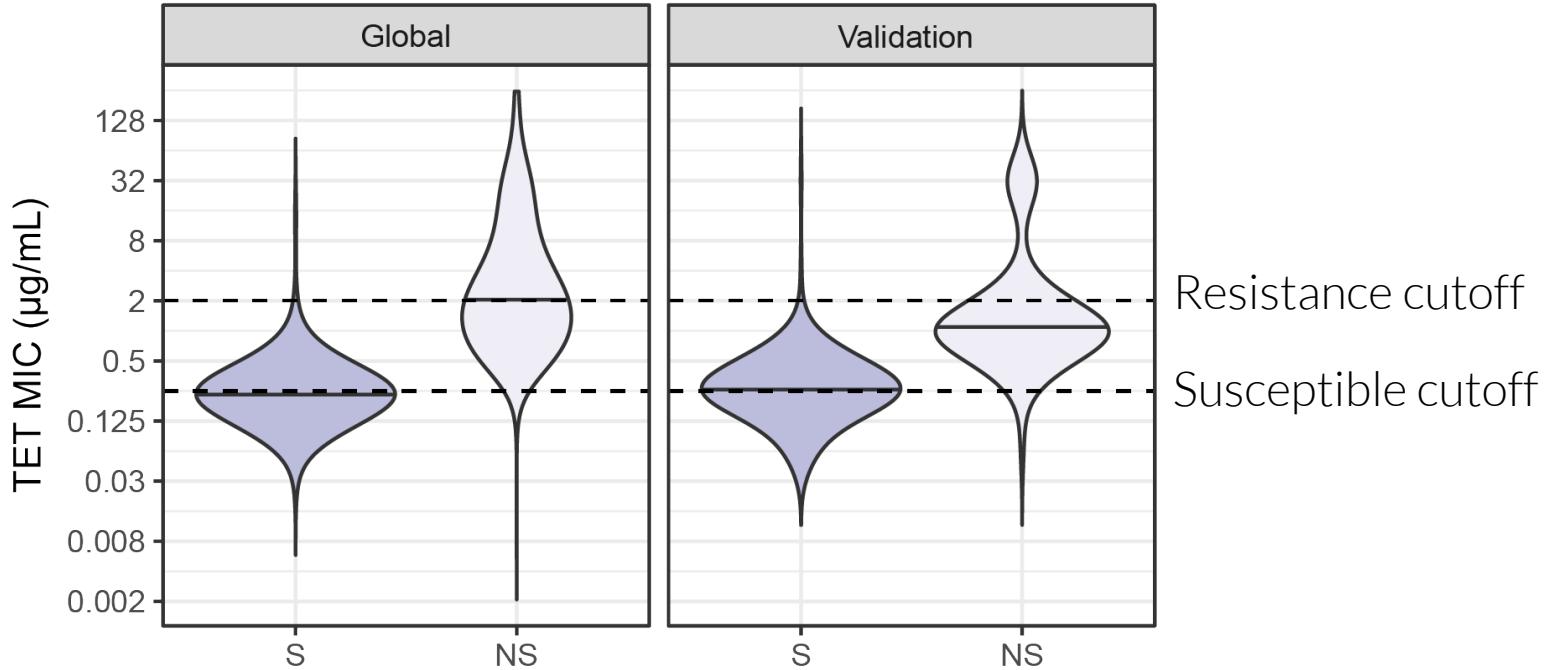
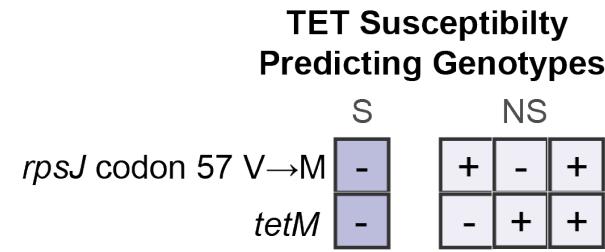
Brian G. Spratt, Lucas D. Bowler, Qian-Yun Zhang,* Jiaji Zhou, and John Maynard Smith

Two loci predict penicillin susceptibility with high specificity



	Global Dataset	Validation Dataset
Sensitivity	36.7%	63.6%
Specificity	99.8%	98.9%

Two loci predict tetracycline susceptibility with high specificity

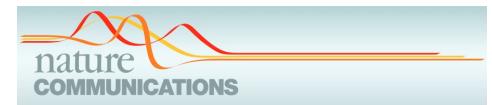


	Global Dataset	Validation Dataset
Sensitivity	88.7%	78.2%
Specificity	97.2%	94.9%

Part 2: Conclusions

- Two loci predict penicillin and tetracycline susceptibility with high specificity but moderate sensitivity
- Additional loci may increase sensitivity
- Conditional GWAS focused on variants associated with susceptibility may be a promising approach to identify loci for molecular diagnostics and surveillance

Part 3: Adaptation to anatomical site of infection selects for increased susceptibility



Adaptation to the cervical environment is associated with increased antibiotic susceptibility in *Neisseria gonorrhoeae*

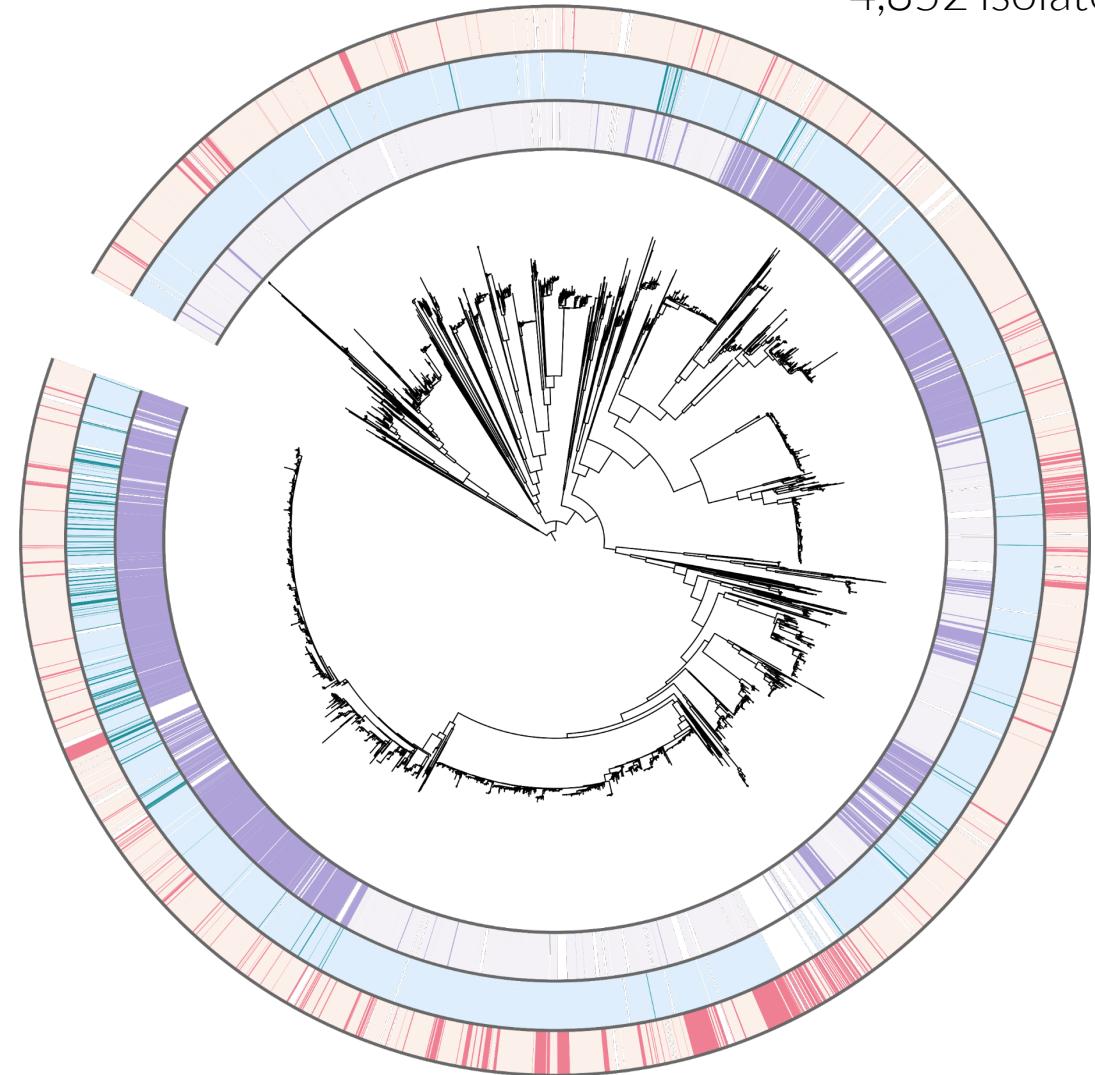
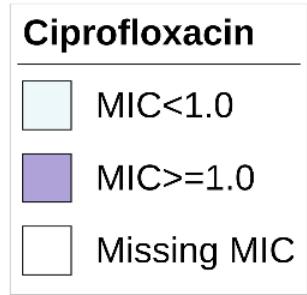
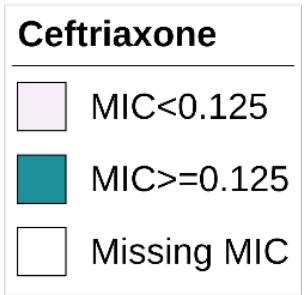
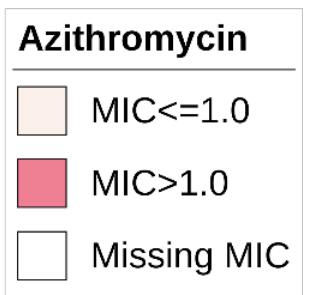
Kevin C. Ma^{1,7}, Tatum D. Mortimer^{1,7}, Allison L. Hicks¹, Nicole E. Wheeler^{1,2}, Leonor Sánchez-Busó^{1,2}, Daniel Golparian^{1,3}, George Taiaroa⁴, Daniel H. F. Rubin¹, Yi Wang¹, Deborah A. Williamson^{1,4}, Magnus Uhemo³, Simon R. Harris⁵ & Yonatan H. Grad^{1,6,✉}

Clinical *N. gonorrhoeae* isolates sometimes revert from antibiotic resistance to susceptibility

4,852 isolates

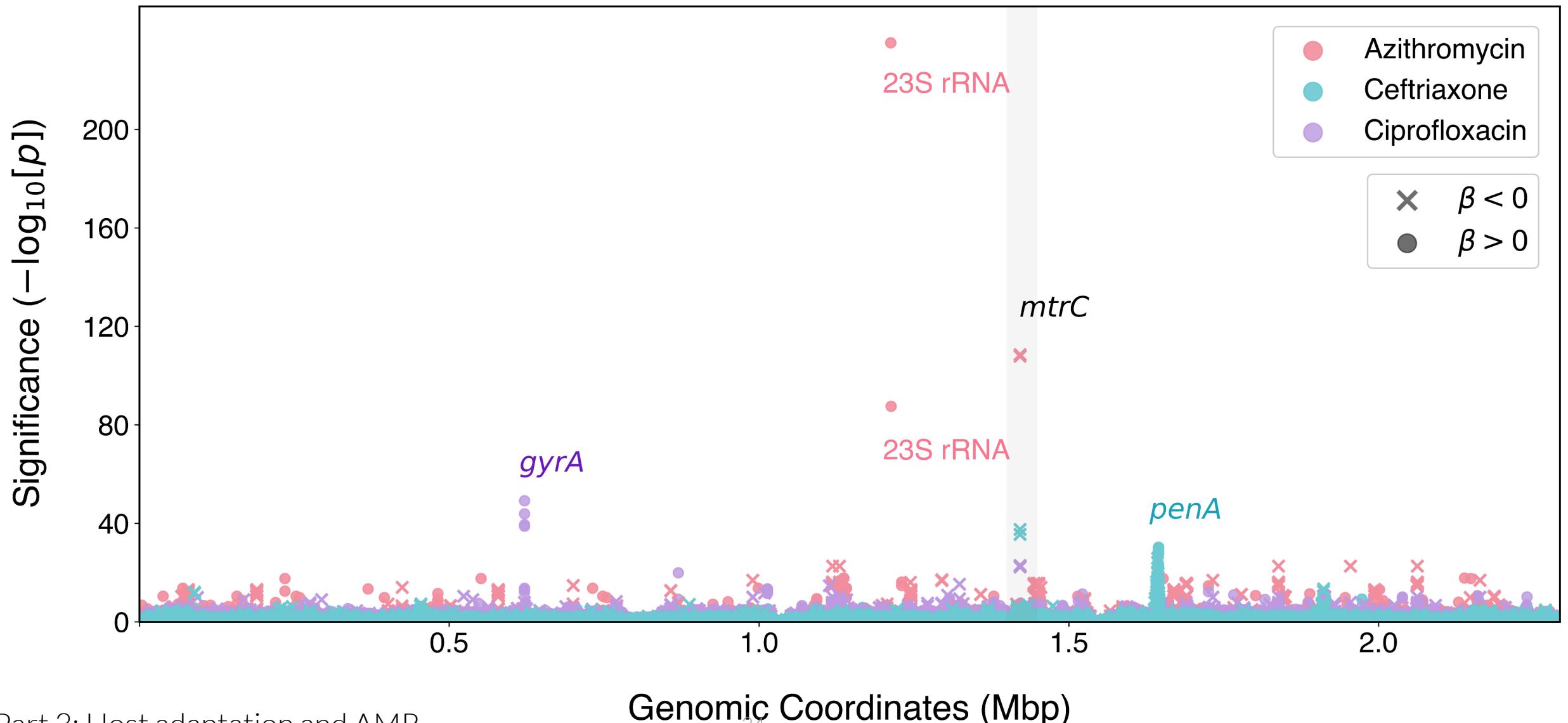
Unexplained susceptibility:

- Azithromycin: 5.7% of strains
- Ceftriaxone: 2.0% of strains
- Ciprofloxacin: 2.9% of strains



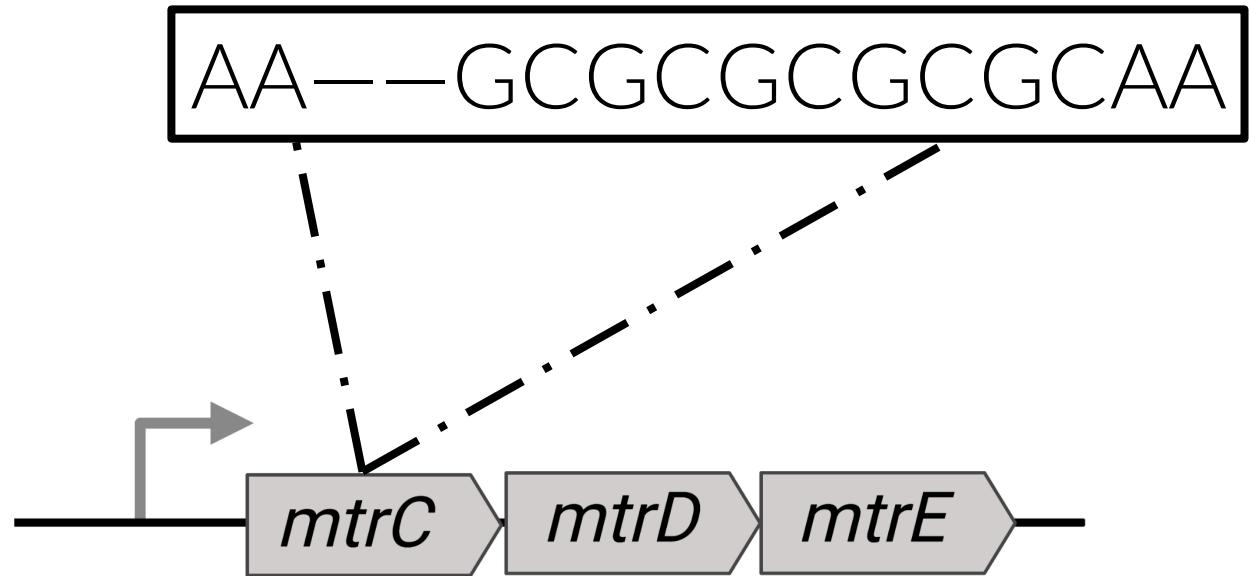
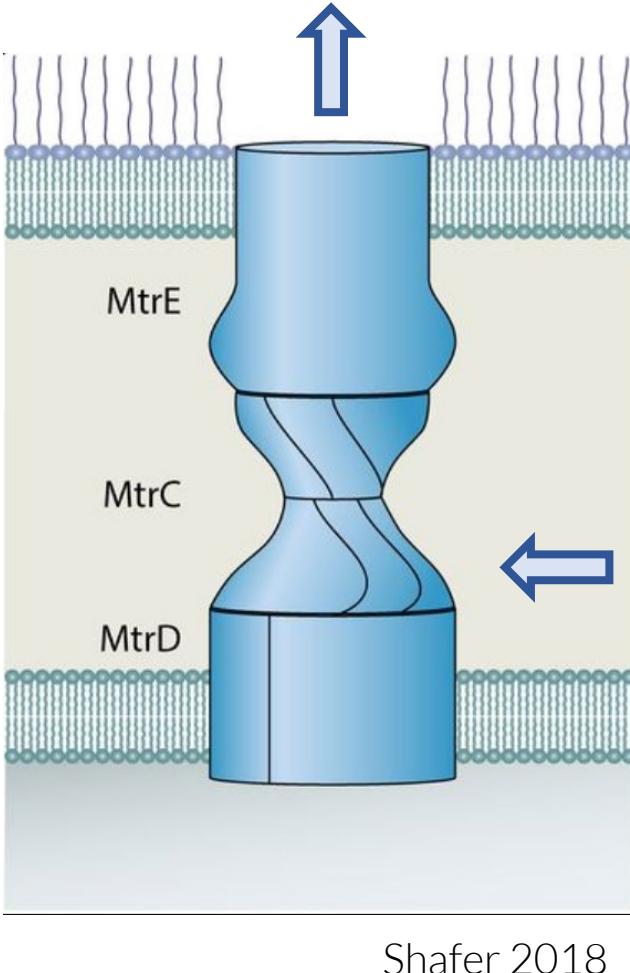
Part 3 Goal:
Identify genetic mechanisms of and
selective pressures for increased susceptibility

GWAS on 4852 strains recovers known resistance genes and identifies an efflux pump mutation associated with multi-drug susceptibility



The significant variant is a two base-pair deletion in the efflux pump component gene *mtrC*

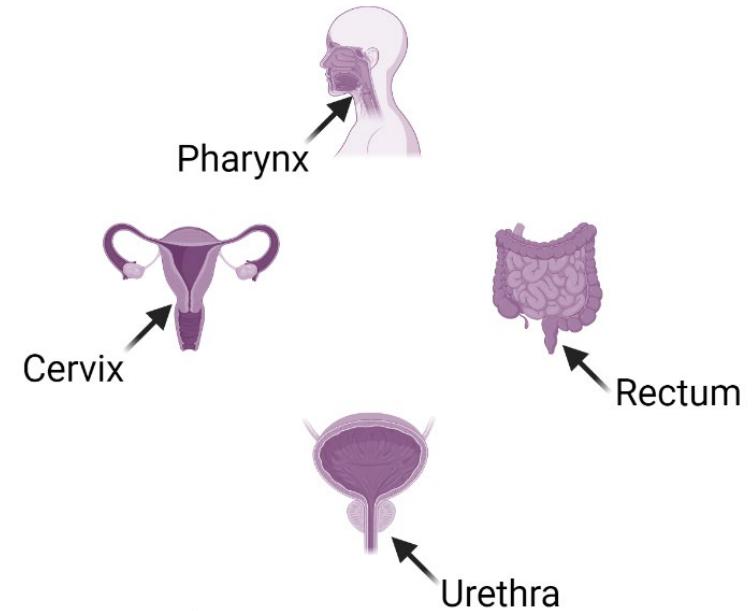
antibiotics, fatty acids, etc.



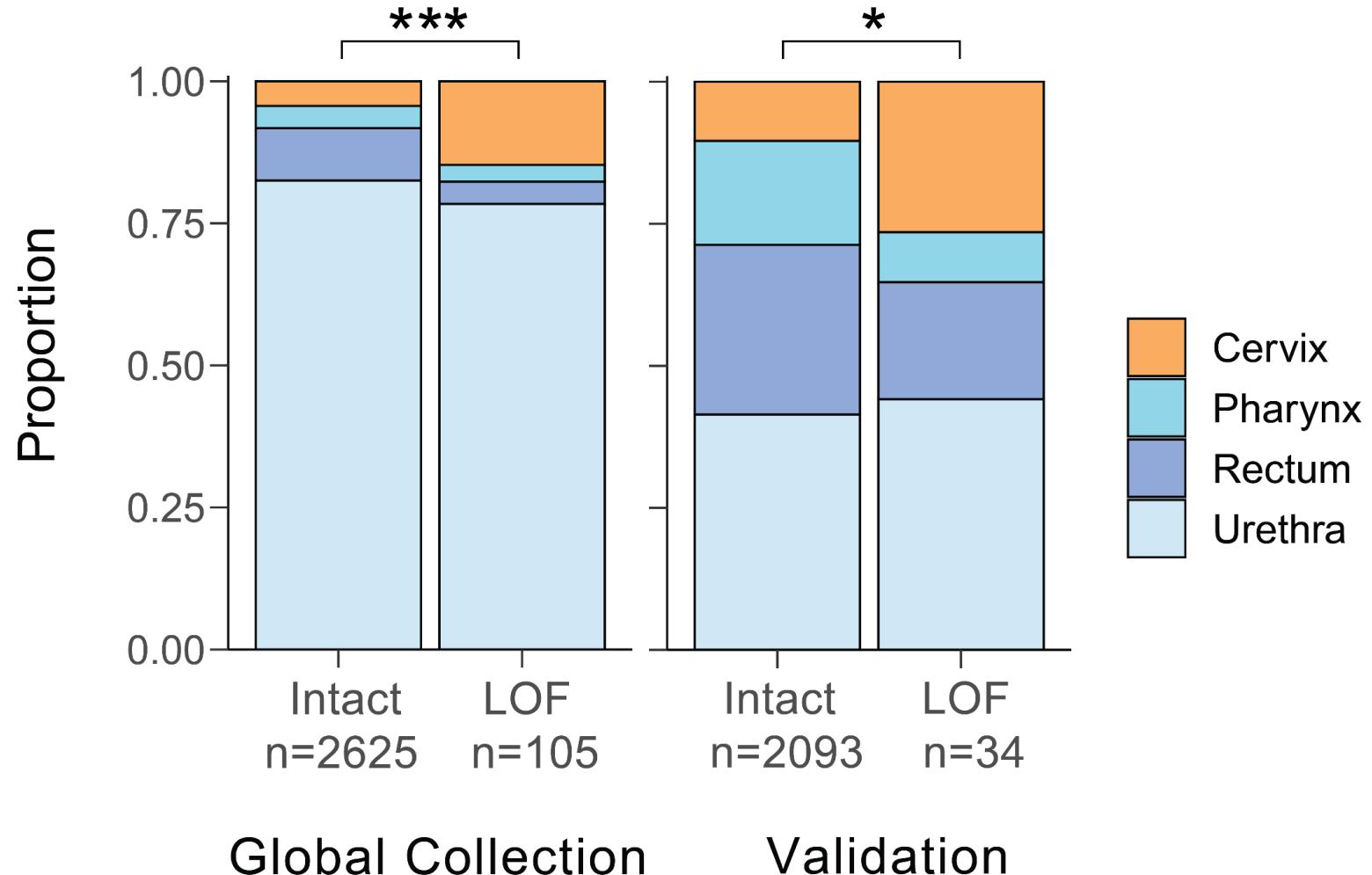
Leads to frameshift and loss-of-function (LOF)

Neisseria gonorrhoeae infection

- Primary infection sites differ in:
 - Oxygen content
 - Iron availability
 - Acidity
 - Microbiota
 - Immunological pressures
 - Antibiotic diffusion



mtrC LOF mutations are overrepresented in cervical infections



*p < 0.05

**p < 0.01

***p < 0.001

Prior work: overexpression of proton-dependent efflux pumps in other species results in fitness costs under anaerobic conditions due to cytoplasmic acidification

Hypothesis: Cervical infection (microaerophilic environment, high acidity) selects for loss of proton-dependent efflux pump activity in *N. gonorrhoeae*

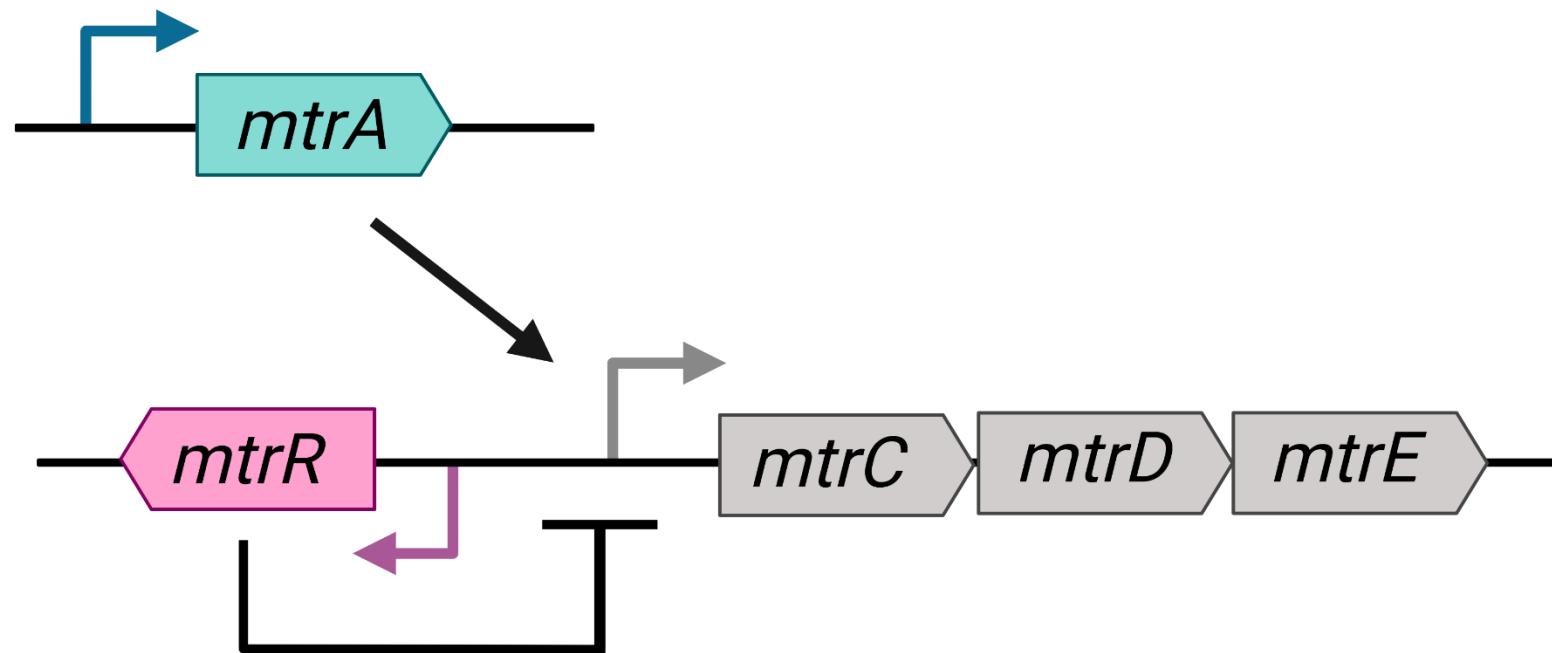
How can we test this hypothesis computationally?

Show that the association of pump inactivation and cervical infection also holds true for:

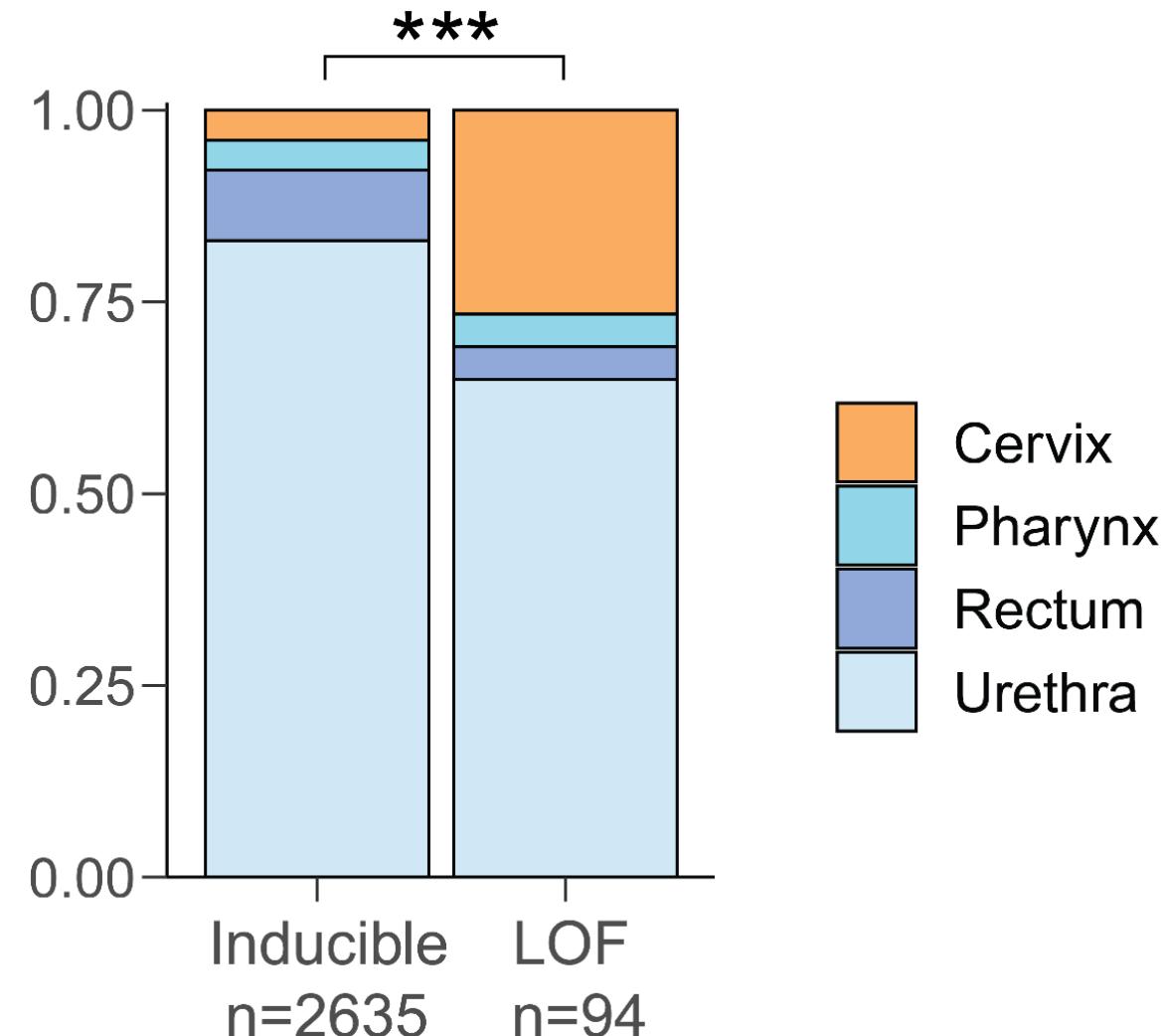
- 1) An activator of MtrCDE
- 2) Other proton-dependent efflux pumps
- 3) Other species that cause urogenital infection

MtrA, the inducible activator of *mtrCDE*, exists in two forms: wild-type and loss-of-function

Induction by low levels of drug



mtrA LOF (n=268; 5.49% of all strains) mutations are also associated with cervical infection



* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

How can we test this hypothesis computationally?

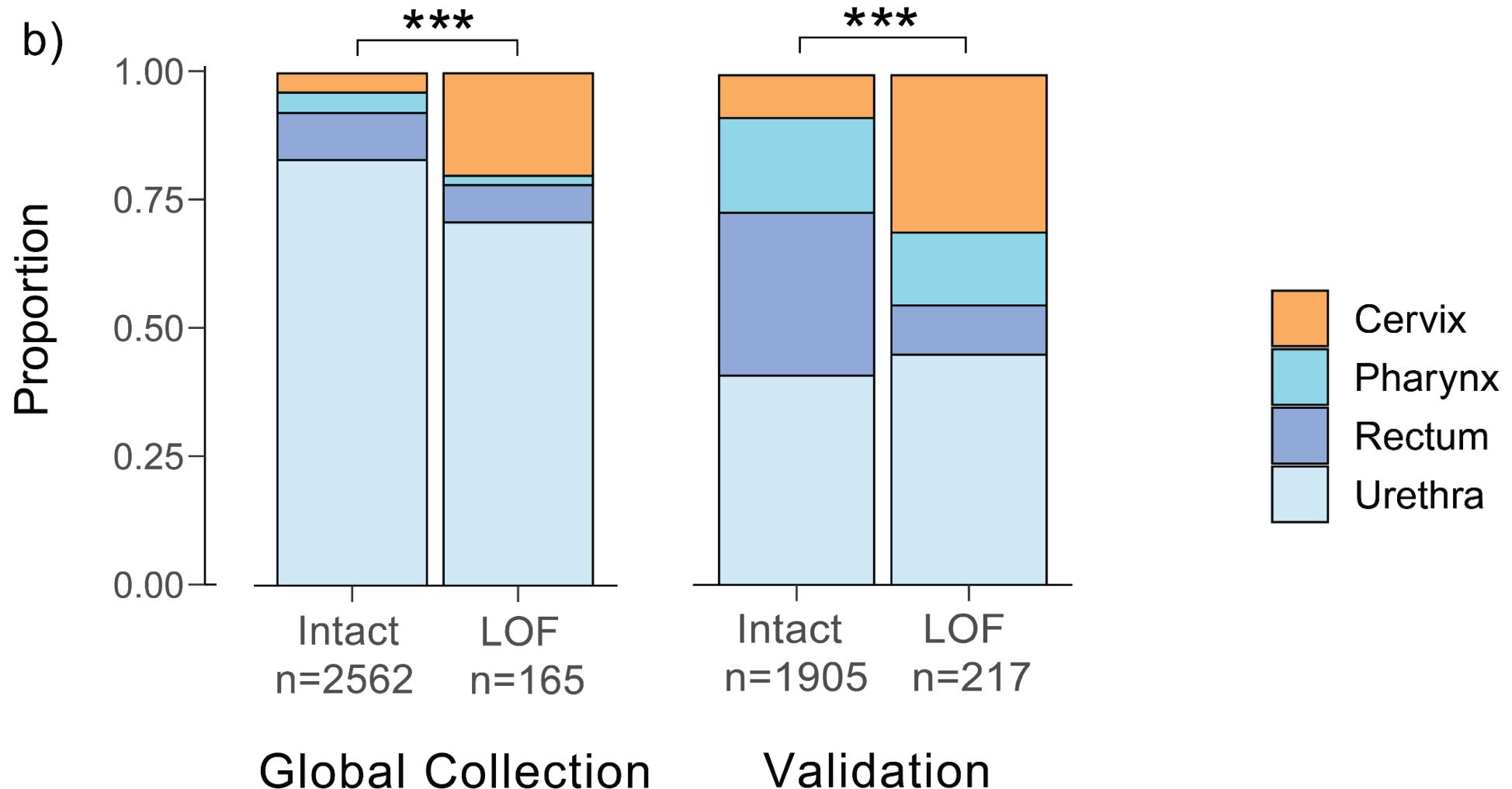
Show that the association of pump inactivation and cervical infection also holds true for:

- 1) An activator of MtrCDE ✓
- 2) Other proton-dependent efflux pumps
- 3) Other species that cause urogenital infection

LOF mutations are observed only in the other gonococcal proton-dependent efflux pump FarAB

Gene	Energy Source	# LOF	Percentage
<i>farA</i>	H+	332	6.86%
<i>farB</i>	H+	2	0.04%
<i>norM</i>	Na+	2	0.04%
<i>macA</i>	ATP	1	0.02%
<i>macB</i>	ATP	13	0.27%

LOF mutations in *farA* are also associated with cervical infection



* $p < 0.05$

** $p < 0.01$

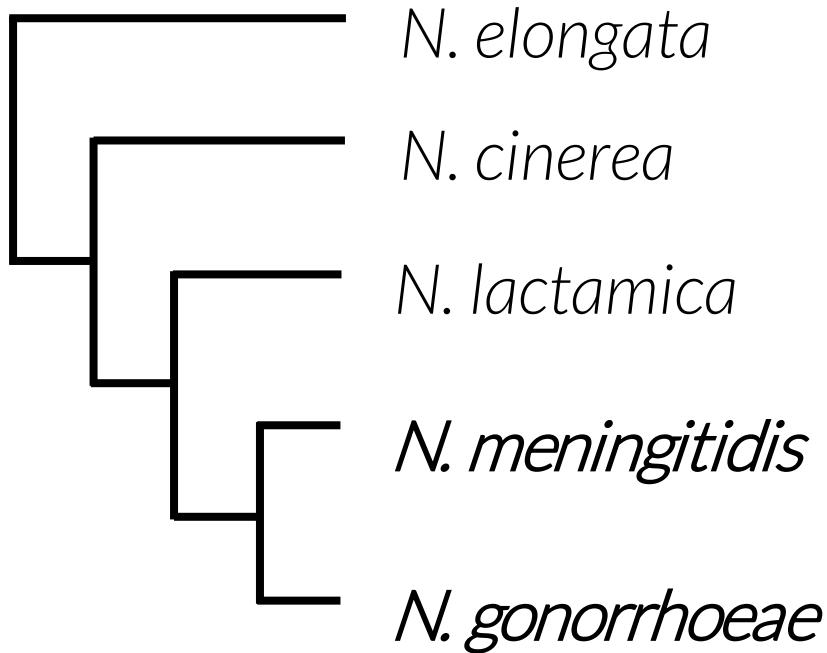
*** $p < 0.001$

How can we test this hypothesis computationally?

Show that the association of pump inactivation and cervical infection also holds true for:

- 1) An activator of MtrCDE ✓
- 2) Other proton-dependent efflux pumps ✓
- 3) Other species that cause urogenital infection

Neisseria meningitidis is a sister species of *N. gonorrhoeae* adapted for pharyngeal colonization



**0.6% (82/14798) of
N. meningitidis
strains have the
mtrC LOF mutation**

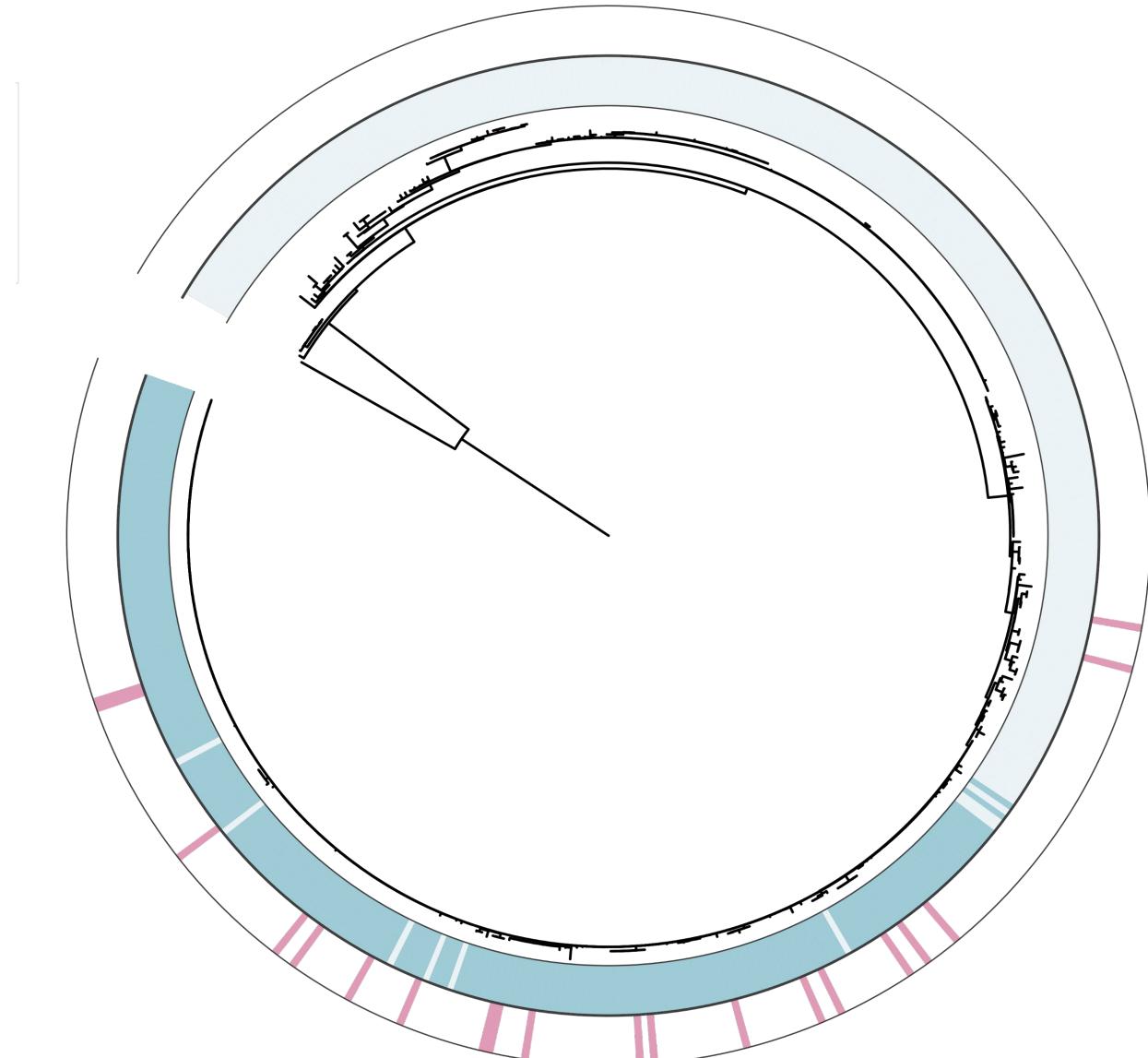
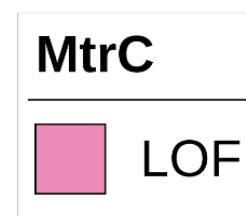
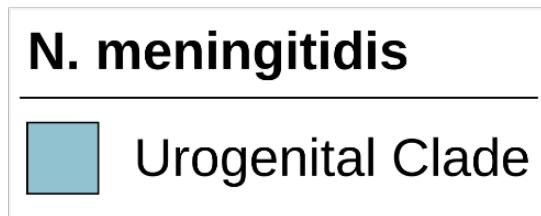
A *N. meningitidis* lineage has adapted for heterosexual urogenital infection by acquiring *N. gonorrhoeae* alleles

Emergence of a new *Neisseria meningitidis* clonal complex 11 lineage 11.2 clade as an effective urogenital pathogen

Yih-Ling Tzeng, Jose A. Bazan, Abigail Norris Turner, Xin Wang, Adam C. Retchless, Timothy D. Read, Evelyn Toh, David E. Nelson, Carlos Del Rio, and David S. Stephens

The urogenital *N. meningitidis* lineage shows enrichment of *mtrC* LOF

18/207 (8.70%) urogenital isolates have *mtrC* LOF vs. 2/248 (0.81%) controls



How can we test this hypothesis computationally?

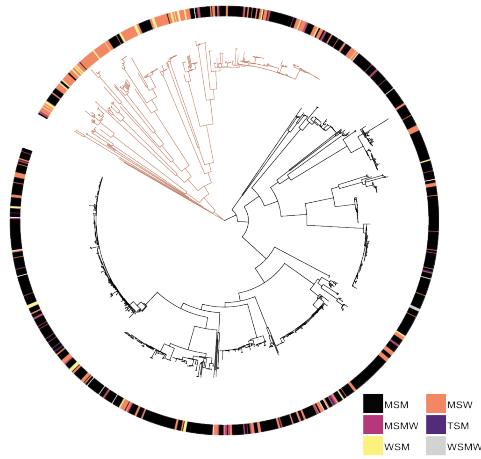
Show that the association of pump inactivation and cervical infection also holds true for:

- 1) An activator of MtrCDE ✓
- 2) Other proton-dependent efflux pumps ✓
- 3) Other species that cause urogenital infection ✓

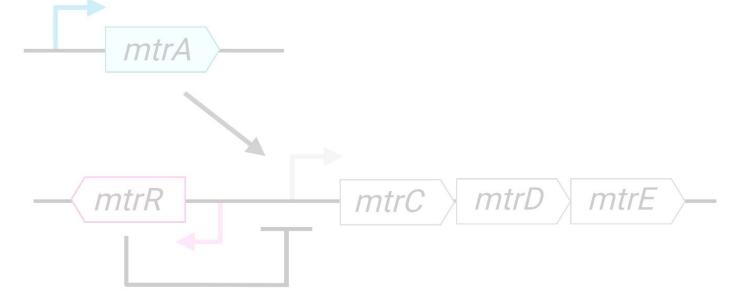
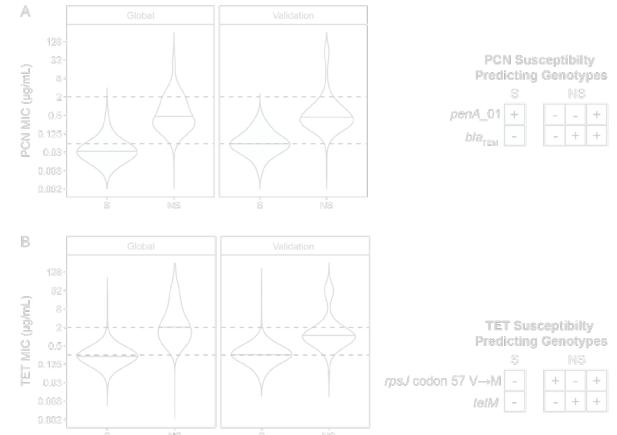
Part 3: Conclusions

- Loss of function mutations in *mtrC* increase susceptibility to multiple antibiotics in *N. gonorrhoeae*
- Adaptation to cervical infections selects for LOF of proton-dependent efflux pumps

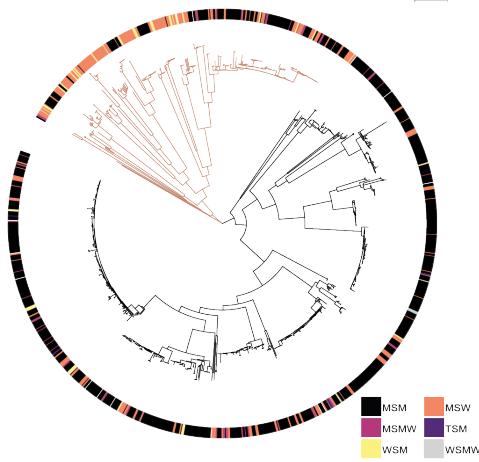
Summary



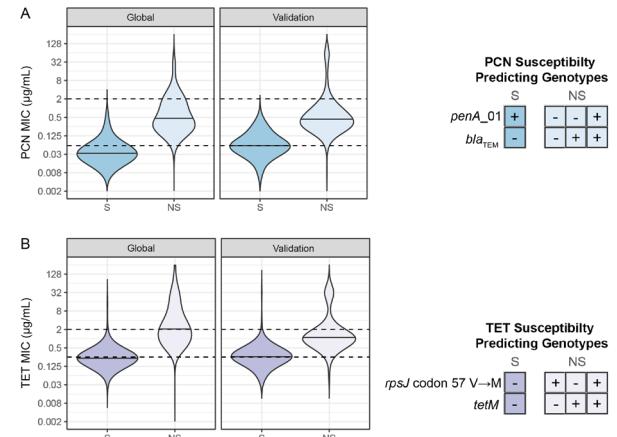
N. gonorrhoeae population is shaped by sexual behavior and AMR



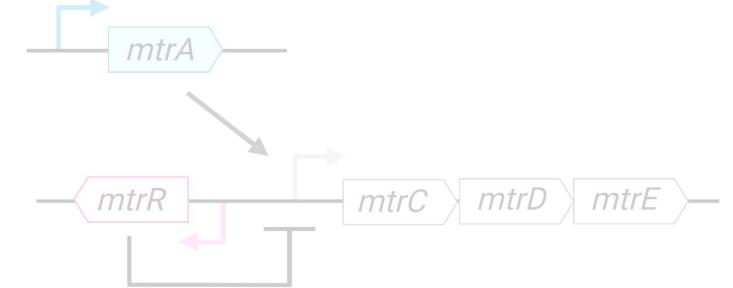
Summary



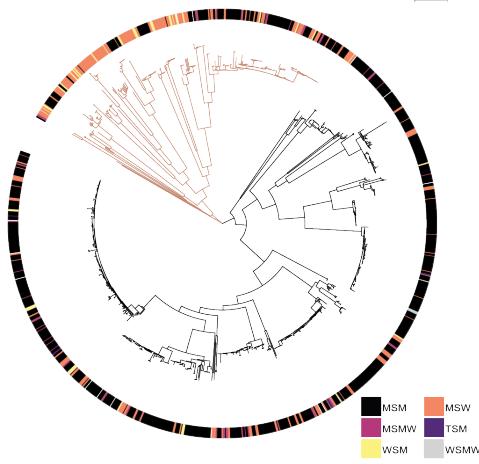
N. gonorrhoeae population is shaped by sexual behavior and AMR



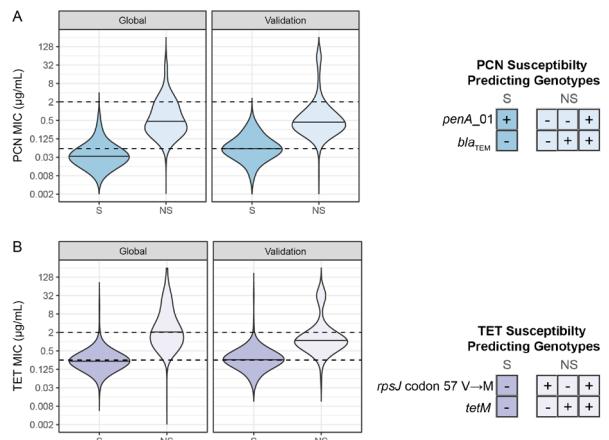
GWAS can identify targets for antimicrobial susceptibility diagnostics



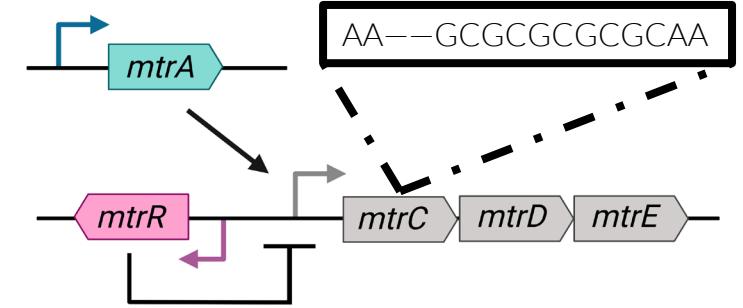
Summary



N. gonorrhoeae population is shaped by sexual behavior and AMR



GWAS can identify targets for antimicrobial susceptibility diagnostics

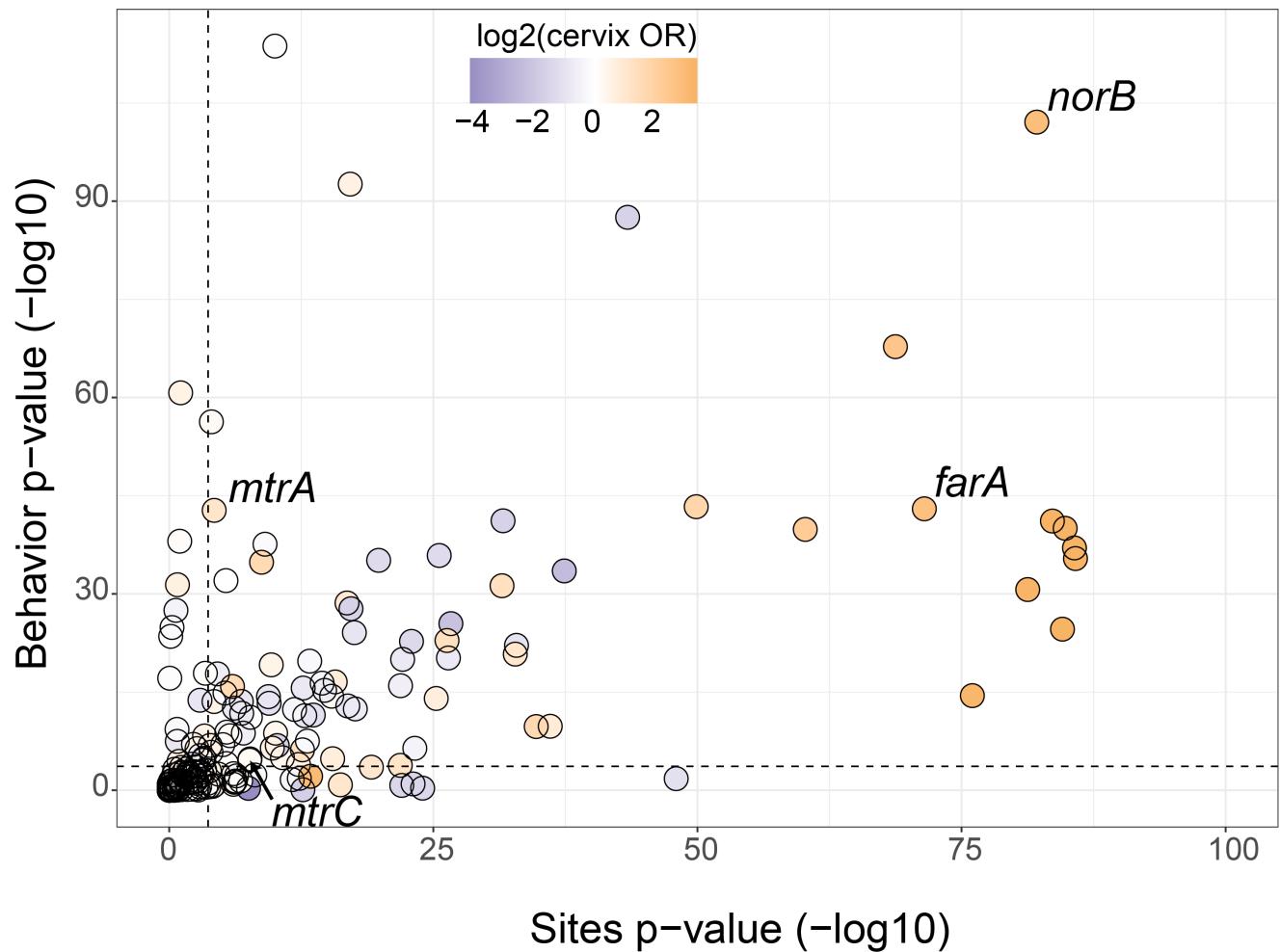


Adaptation to anatomical site of infection selects for increased susceptibility

Future Directions in Host Adaptation and AMR

237 genes with prevalent LOF alleles!

LOF is associated with adaptation to sexual behavior and site of infection

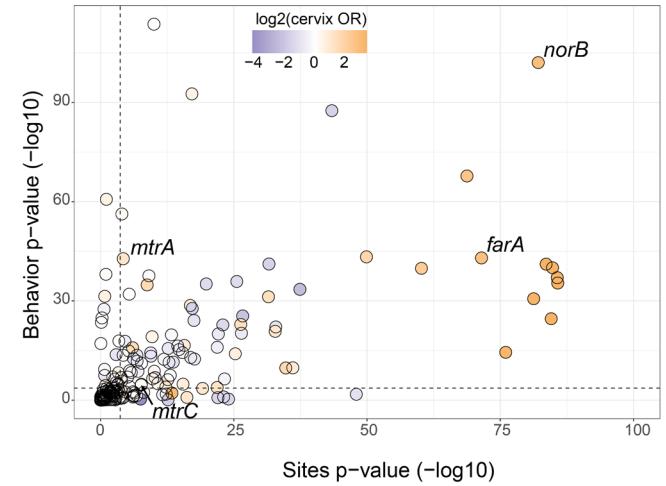


Future Directions in Host Adaptation and AMR

How are LOF alleles
gained and lost?
Mutation?
Recombination?

Is LOF a common
mechanism of
adaptation in
bacterial pathogens?

What is the role of
within-host
diversity?



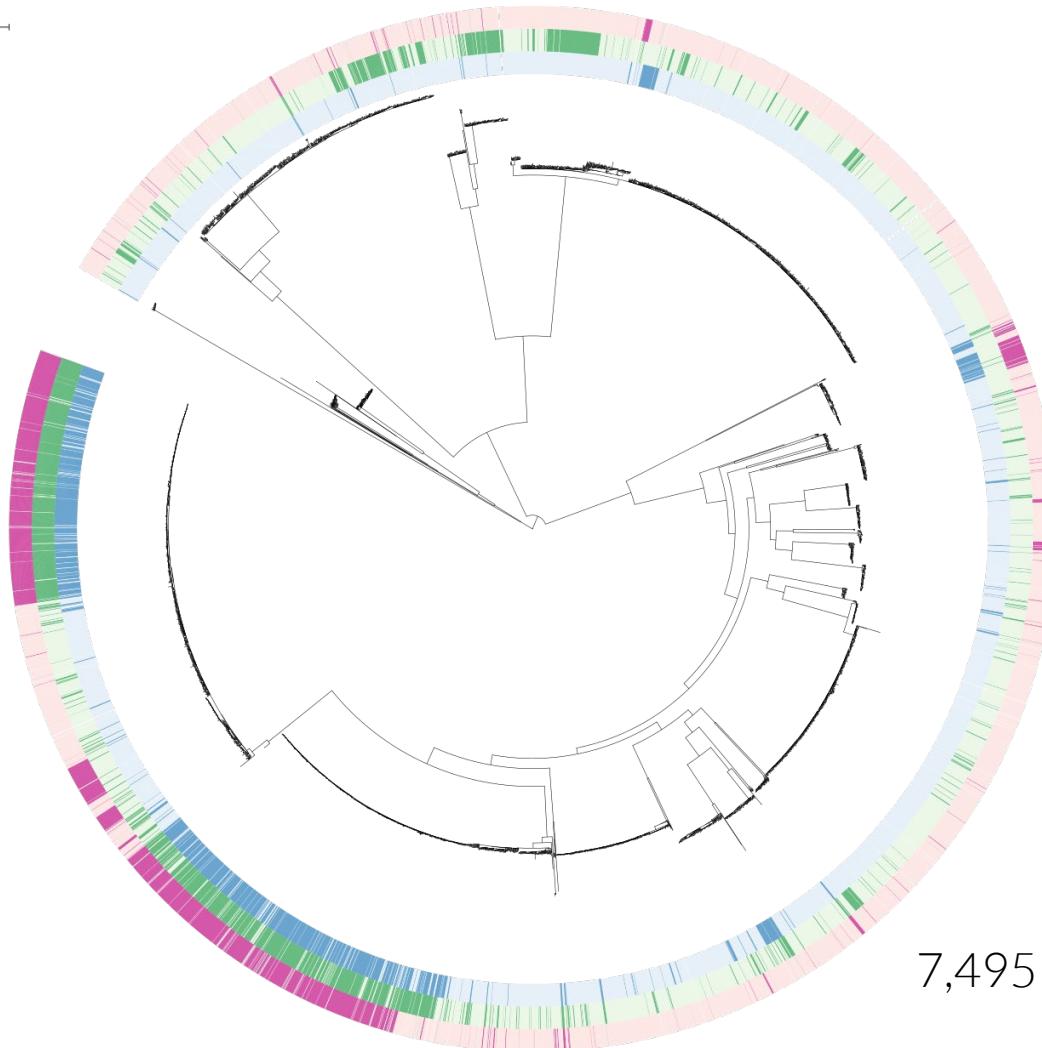
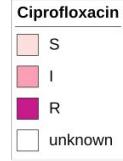
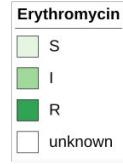
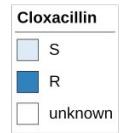
Evolutionary mechanisms

Associated variants

Improved interventions,
diagnostics, and treatments

Future Directions in Host Adaptation and AMR

Tree scale: 0.01



7,495 *Staphylococcus aureus* isolates

LSARP Project:

- 30,000+ bacteremia isolates from Alberta, Canada, 2006-2020
- Dense patient and microbiological metadata
- Genomic, proteomic, and metabolomic data for all isolates

Future Directions in Host Adaptation and AMR

In what environments are susceptibility alleles enriched?

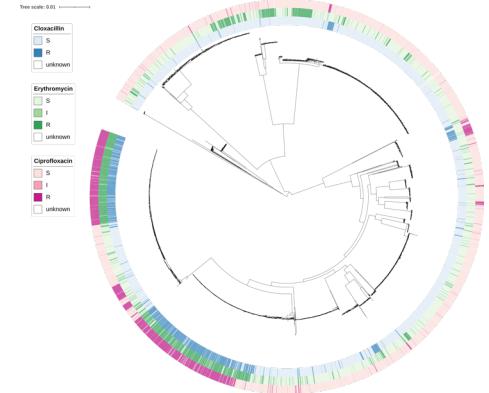
Evolutionary mechanisms

In what genetic context do we find susceptibility associated alleles?

Associated variants

Can we identify variation associated with susceptibility in other pathogens?

Improved surveillance, diagnostics, & interventions



Acknowledgments

Grad Lab

Yonatan Grad
Aishani Aatresh
Adi Bandekar
Lisa Frielinghaus
Stephen Kissler
Maddy Kline
Tse Yang Lim
Rebecca McSweeney
Jason Olejarz
Sam Palace
Mui Pham
Emily Reichert
Kirstin Roster
Dan Rubin
QinQin Yu



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Figures from BioRender.com



F32AI145157

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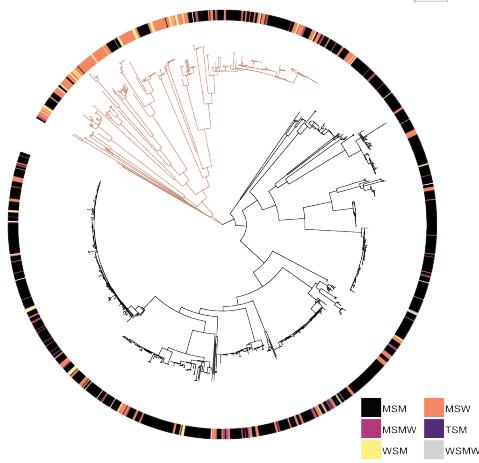
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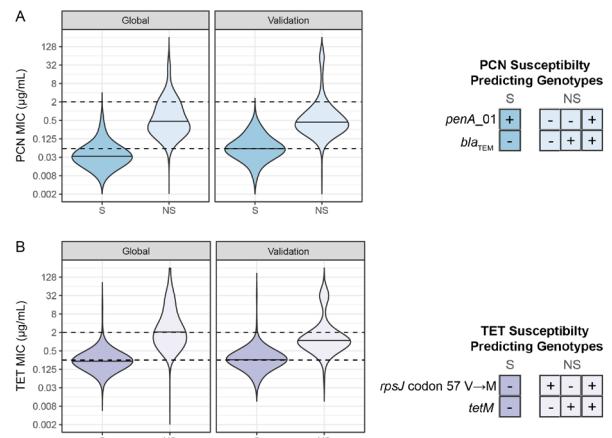
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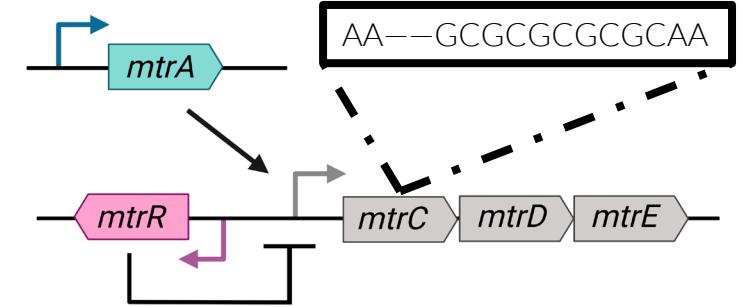
Summary



N. gonorrhoeae population is shaped by sexual behavior and AMR

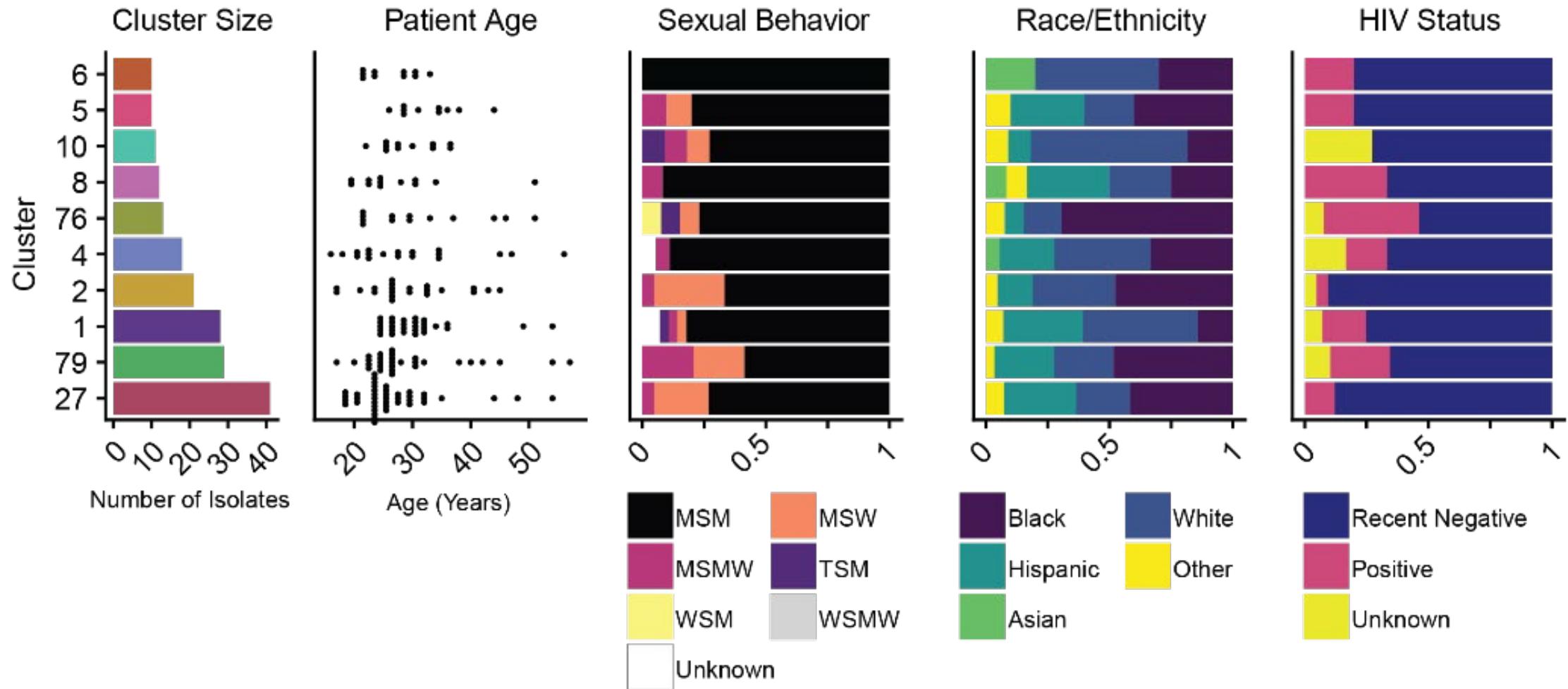


GWAS can identify targets for antimicrobial susceptibility diagnostics

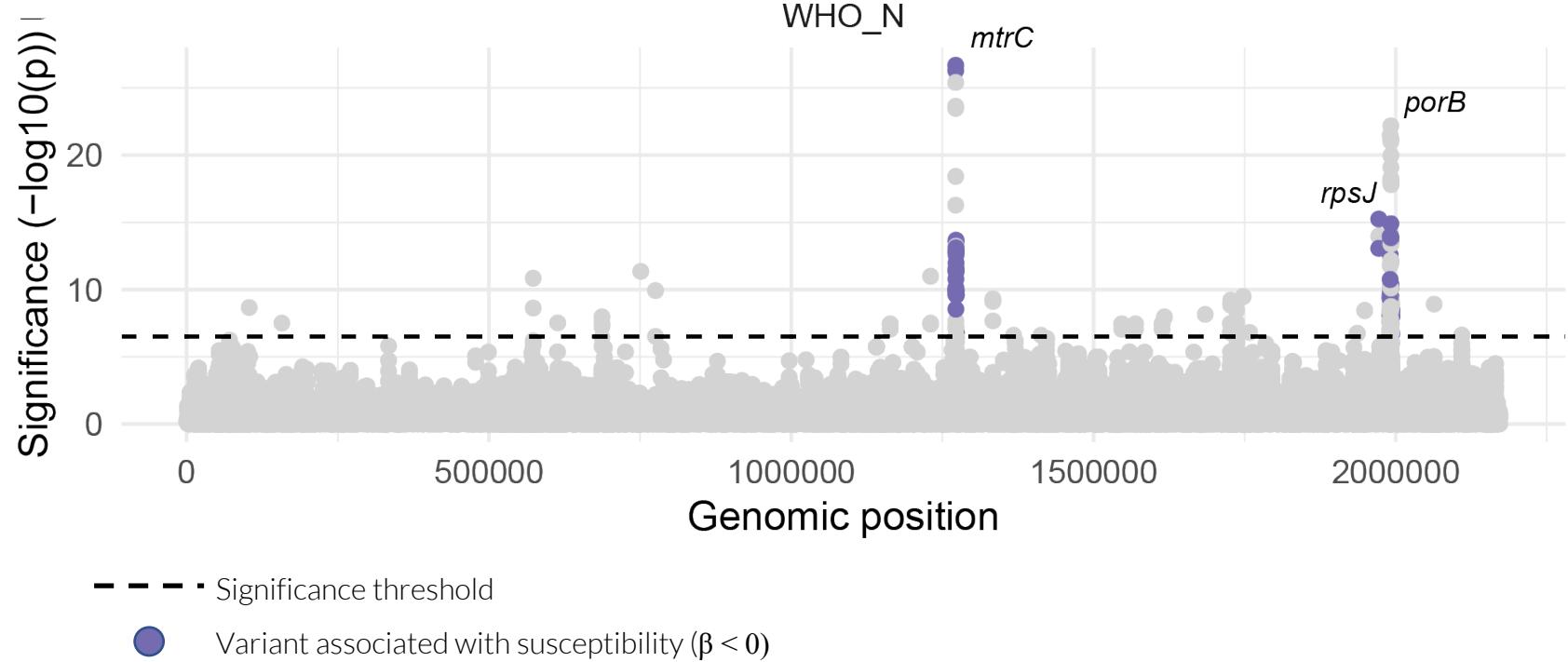
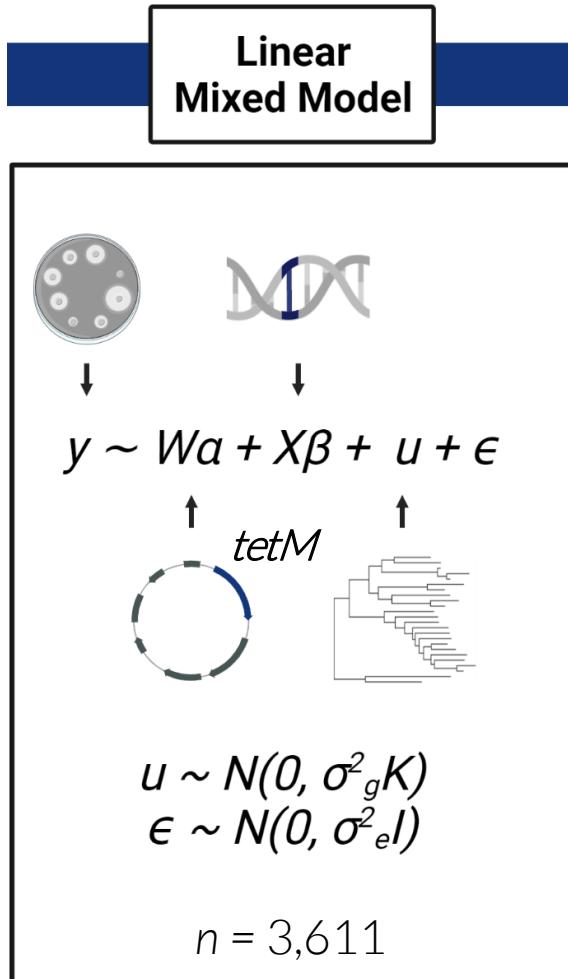


Adaptation to anatomical site of infection selects for increased susceptibility

In largest clusters, transmission across demographic groups



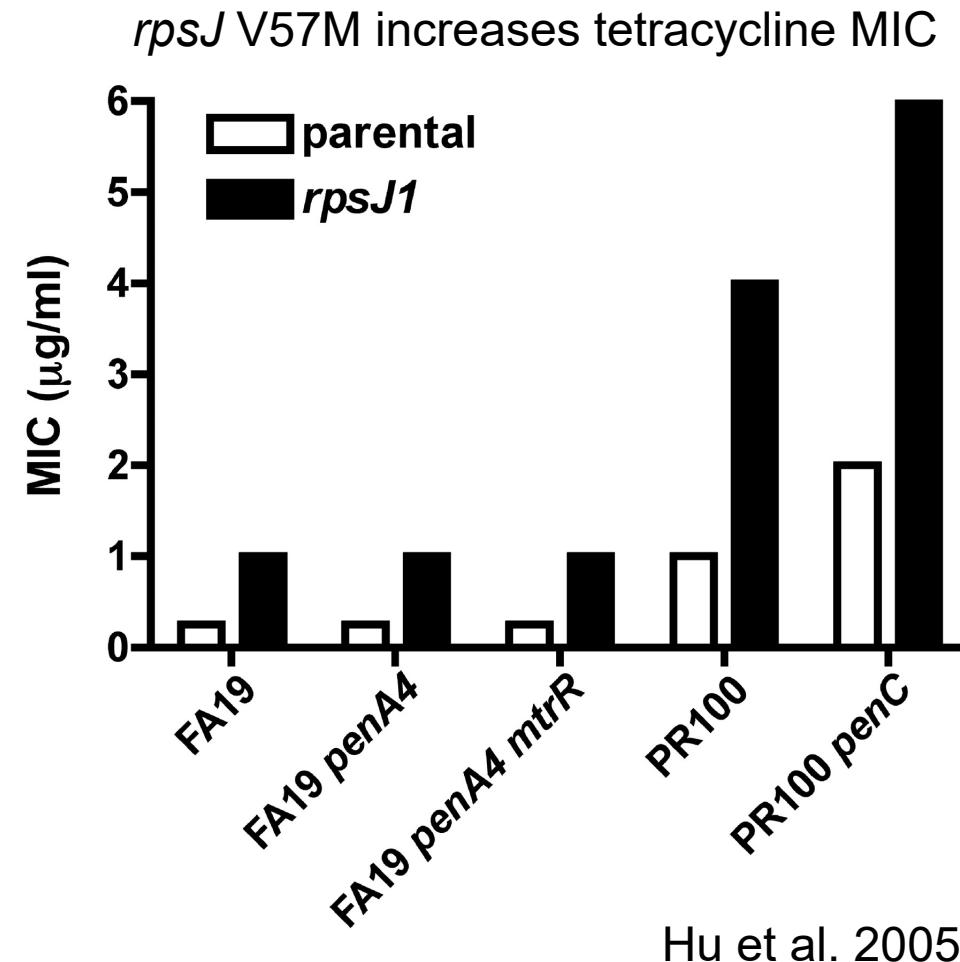
Tetracycline conditional GWAS



rpsJ unitig differentiates susceptible allele from *rpsJ* V57M

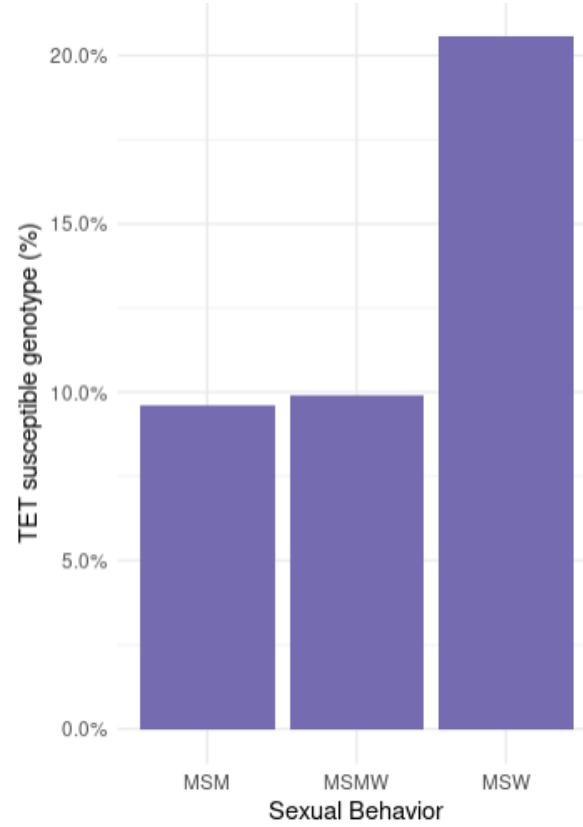
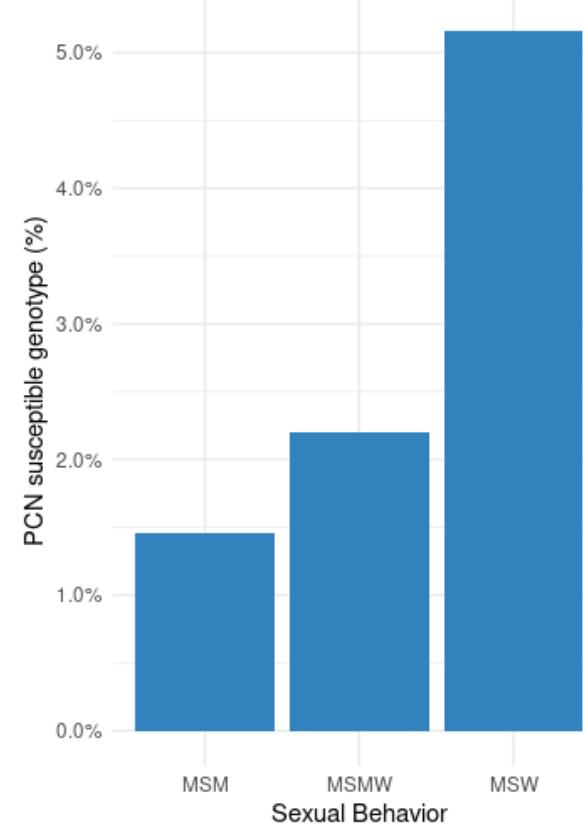
rpsJ WT GAG CG TTT CAAC AT TTT GCG TT CT CCG CAC GT GA AC AAA ACT TCCC GT GA
rpsJ V57M GAG CG TTT CAAC AT TTT GCG TT CT CCG CAC AT GA AC AAA ACT TCCC GT GA

$$p = 5.6 \times 10^{-16}, \beta = -1.61$$



Prevalence varies across datasets and demographics

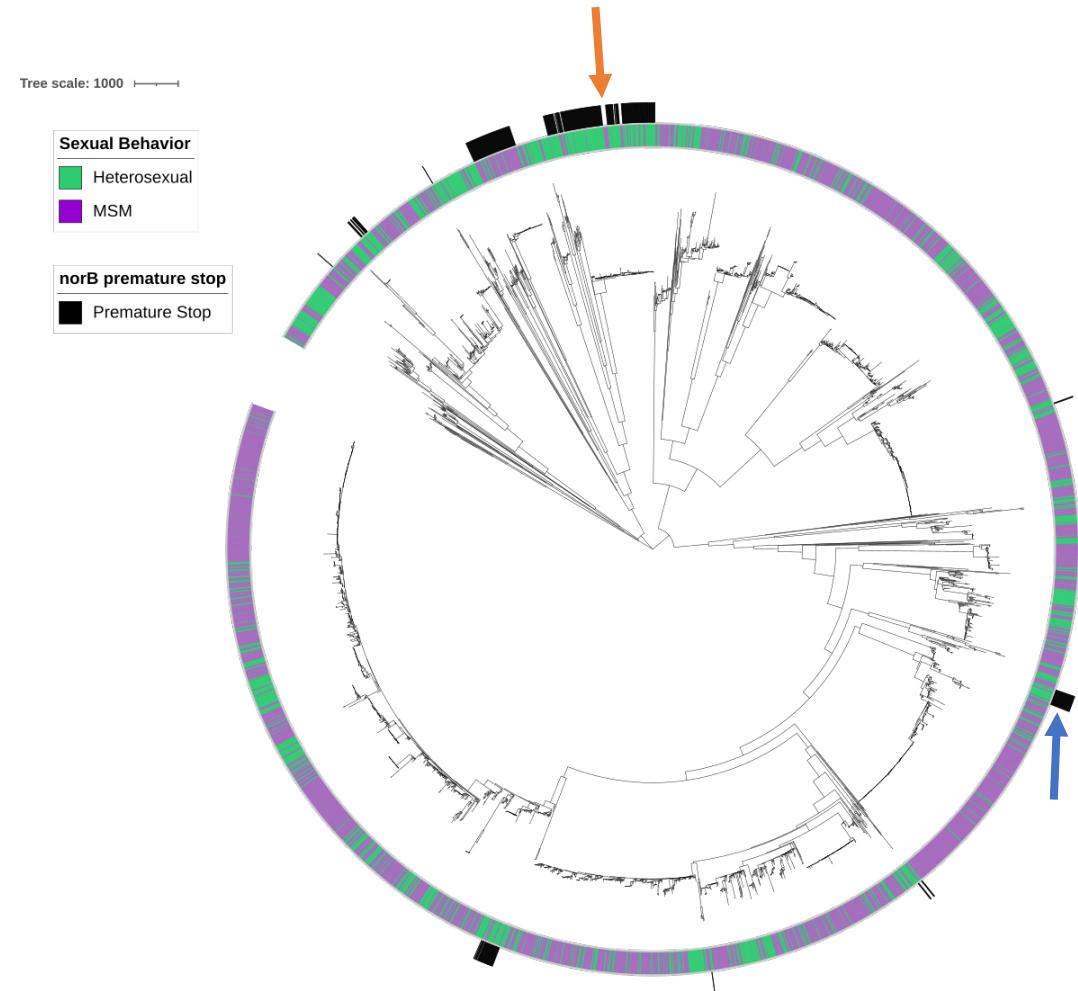
Dataset	PCN susceptible genotype	TET susceptible genotypes
Alfsnes et al. 2020	18 (1.9%)	222 (24.0%)
Buckley et al. 2018	0 (0.0%)	93 (100.0%)
Cehovin et al. 2018	0 (0.0%)	0 (0.0%)
De Silva et al. 2016	35 (2.5%)	219 (15.6%)
Demczuk et al. 2015	7 (6.6%)	20 (18.9%)
Demczuk et al. 2016	3 (1.5%)	1 (0.5%)
Eyre et al. 2017	21 (9.1%)	33 (14.3%)
Ezewudo et al. 2015	3 (5.6%)	8 (14.8%)
Fifer et al. 2018	0 (0.0%)	0 (0.0%)
Grad et al. 2014, 2016	11 (1.0%)	56 (5.1%)
Harris et al. 2018	54 (5.3%)	119 (11.7%)
Kwong et al. 2017	0 (0.0%)	2 (2.1%)
Lan et al. 2020	2 (0.9%)	2 (0.9%)
Lee et al. 2018	4 (1.0%)	119 (30.0%)
Mortimer et al. 2020	34 (3.8%)	129 (14.4%)
Peng et al. 2019	0 (0.0%)	0 (0.0%)
Ryan et al. 2018	0 (0.0%)	1 (2.6%)
Sánchez-Busó et al. 2018	30 (7.9%)	36 (9.5%)
Thomas et al. 2019	7 (1.1%)	59 (9.6%)
Town et al. 2020	13 (1.0%)	257 (20.2%)
Williamson et al. 2019	6 (0.3%)	506 (23.2%)
Yahara et al. 2018	4 (1.5%)	36 (13.8%)



GISP 2018 (Reimche et al. 2021)

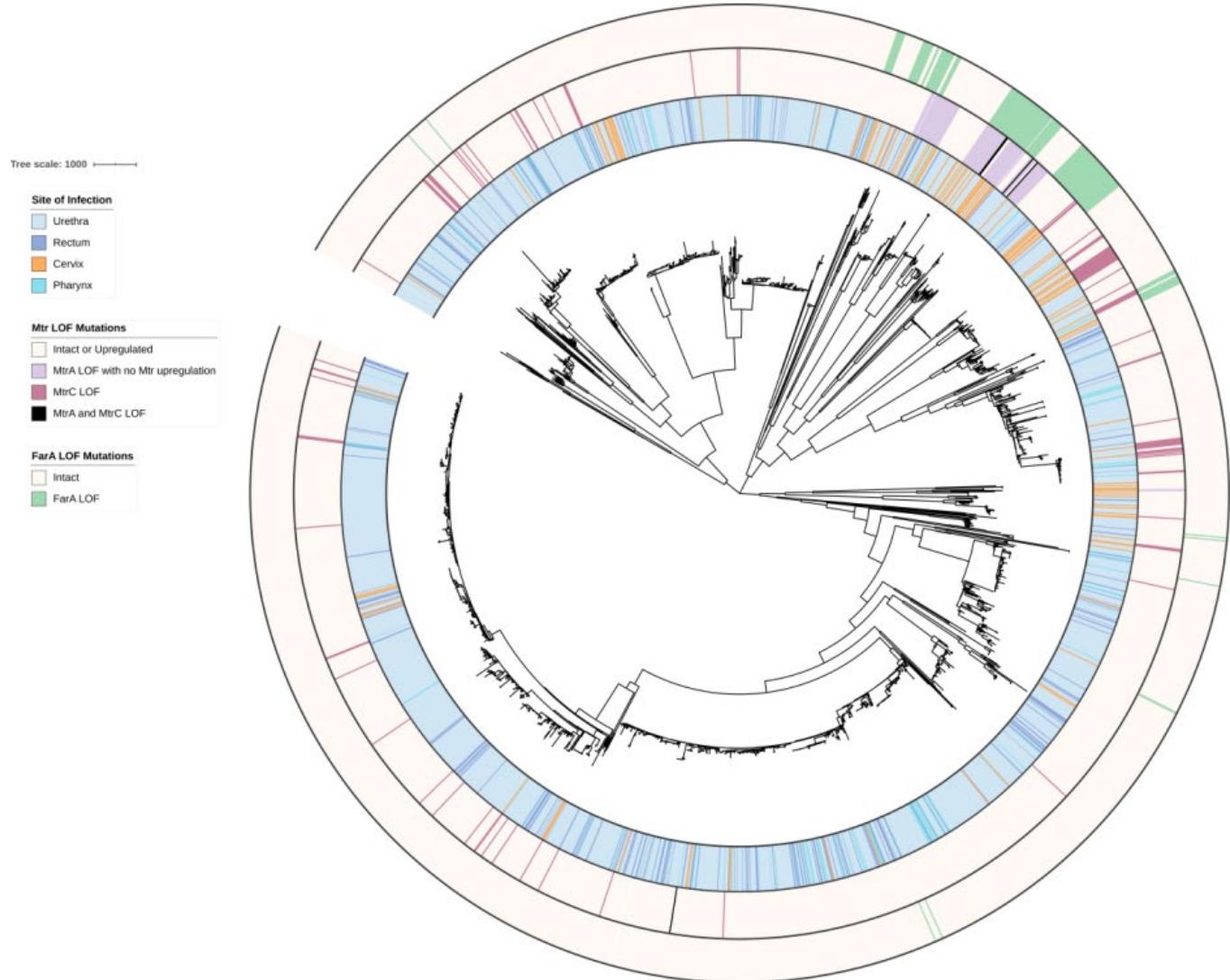
norB loss of function is associated with cervical infections

- 23.3% of cervical isolates encode *norB* premature stop
- For comparison, 3.4% of rectal isolates encode *norB* premature stop
- Frameshifts in *norB* caused by:
 - 1 bp insertion
 - G->A nonsense mutation
 - 14 bp insertion
- Flanking variation suggests that *norB* reading frame is restored by recombination



Genetic background may influence the distribution of LOF alleles

- *mtrC* LOF rarely occurs on the same genetic background as *mtrA* LOF
- *mtrC* LOF occurs primarily in backgrounds with *mtr* upregulation
- *mtrA* LOF occurs primarily in backgrounds without *mtr* upregulation



norB encodes nitric oxide reductase

- Required for anaerobic growth, but not anaerobic survival, *in vitro*
(Householder et al. 2000)
- Nitric oxide produced by *Neisseria gonorrhoeae* suppresses cervical epithelial cell exfoliation
(Muenzer and Hauck 2020)

