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Doxycycline Postexposure Prophylaxis Could Induce Cross-Resistance to Other Classes of Antimicrobials in Neisseria gonorrhoeae: An In Silico Analysis

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Doxycycline Postexposure Prophylaxis Could Induce Cross-Resistance to Other Classes of Antimicrobials in *Neisseria gonorrhoeae*: An In Silico Analysis

Thibaut Vanbaelen, MD, Sheeba Santhini Manoharan-Basil, PhD,* and Chris Kenyon, MD, PhD, MPH*†*

Abstract: We found that tetracycline resistance–associated mutations and genes in *Neisseria gonorrhoeae* are linked to mutations causing resistance to other antimicrobials. Therefore, the use of doxycycline postexposure prophylaxis may select for resistance to other antimicrobials.

Three randomized controlled trials have now established that doxycycline postexposure prophylaxis (PEP) can reduce the incidence of chlamydia and syphilis in men who have sex with men (MSM).[1–3] The Doxycycline Postexposure Prophylaxis (Doxy PEP) study, the largest and most rigorous of these studies, found that doxycycline also reduced the incidence of *Neisseria gonorrhoeae*.[3] As a result of these findings, certain clinics in San Francisco are now offering doxycycline PEP to a proportion of MSM attending their clinics.[4]

A major concern about the widespread use of doxycycline PEP is that it will induce resistance to tetracyclines in *N. gonorrhoeae* and other bacterial species. Two doxycycline PEP studies have evaluated the effect of doxycycline on tetracycline resistance in *N. gonorrhoeae*. Both found no statistically significant effect, but the duration of follow-up was short, and the number of gonococcal isolates tested was small (n = 9 isolates[1] and n = 47 isolates[3]).

An underexplored risk of doxycycline PEP is the selection of resistance to other classes of antimicrobials. The excess use of antimicrobials has been frequently associated with the selection of cross-resistance to related and unrelated classes of antimicrobials in a number of bacterial species.[5] This effect can be direct or indirect. In the direct pathway, tetracyclines have been noted to induce mutations that confer cross-resistance to fluoroquinolones, β-lactams, and other classes of antimicrobials in *Escherichia coli* in vitro.[6,7] Tetracyclines can also act indirectly. If, for example, the genetic determinants of doxycycline resistance in *N. gonorrhoeae* are strongly linked to markers of resistance to other antimicrobials, then the use of doxycycline may indirectly select for resistance to these other antimicrobials. This has been shown for other species, such as the selection for macrolide resistance in *Streptococcus pyogenes*.[8]

To test this indirect-pathway hypothesis, we assessed the extent to which tetracycline-resistance-associated mutations (RAMs) were clonally distributed in *N. gonorrhoeae* and if these RAMS were associated with resistance-conferring mutations to other classes of antimicrobials.

We tested the 2 major determinants of reduced susceptibility to tetracyclines—*tetM* and *rpsJ* V57M. High-level tetracycline resistance (>16 mg/L) is typically due to the plasmid-mediated acquisition of the *tetM* gene.[9] The *rpsJ* V57M substitution reduces the affinity of the 30S ribosome subunit for tetracyclines and results in lower-level resistance.[9]

MATERIALS AND METHODS

N. gonorrhoeae Collection

We analyzed the 2375 gonococcal isolates from the 2018 Euro-GASP survey (https://pathogen.watch/collection/eurogasp2018). This survey collected the samples from individuals who had culture-positive gonococcal infection episodes in 26 European Union and European Economic Area countries via a validated sampling methodology.[10,11] Whole-genome sequencing was performed, and genogroups and AMR determinants were deduced from quality-checked genomic data.[10]

DATA ANALYSIS

All known RAMs were grouped per gene to construct a binary variable per gene that indicated if any RAM was present in that isolate. For *gyrA*, for example, if any of the known GyrA RAMs were present (S91F, D95A, D95G, D95N), the GyrA variable was coded as 1 and coded as 0 if no RAMs were found. The RAMs used to construct the variables are as follows: *gyrA* (S91F, D95A, D95G, D95N), *parC* (D86N, S88P, E91K), *penA* (A311V, V316T, I312M, ins346D, T483S, P551S, G542S, G545S), *ponA* (L421P), *porB1a* (G120K, G120D/A121D), mtr promoter (a57del), and *folP* (R228S).[10] To assess for clonality, we assessed the prevalence of *rpsJ* V57M and *tetM* by genogroup. This analysis was limited to the genogroups with more than 50 isolates. Statistical analyses were conducted using Stata V16 and the χ^2 test to compare groups.

RESULTS

Clonality by Genogroup

We found strong evidence of clonal spread of *rpsJ* V57M and *tetM* by genogroup (Fig. 1).

rpsJ

In 7 of the 11 genogroups with more than 50 isolates, all the isolates had the *rpsJ* V57M mutation (n = 592). For the 4

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Ethics Statement: This analysis involved a secondary data analysis of anonymized public-access data.
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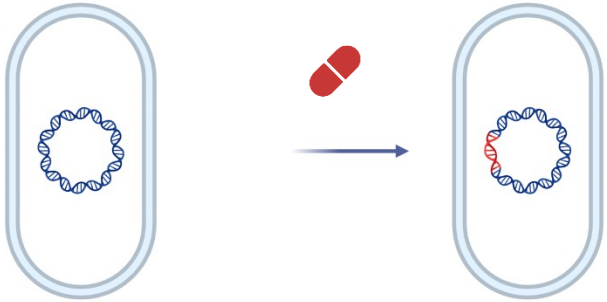
Introduction

- Three RCTs have shown that doxyPEP is effective in reducing the incidence of Chlamydia and Syphilis in MSM and TGW
- DoxyPEP can result in very high doxycycline consumption
- There are concerns doxyPEP can induce doxycycline resistance in *Neisseria gonorrhoeae* (Ng)
- There is also a risk of inducing AMR to other classes of antimicrobials in Ng

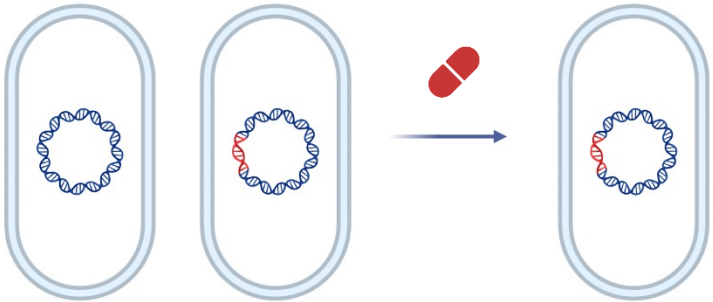
Cross resistance

Direct pathway

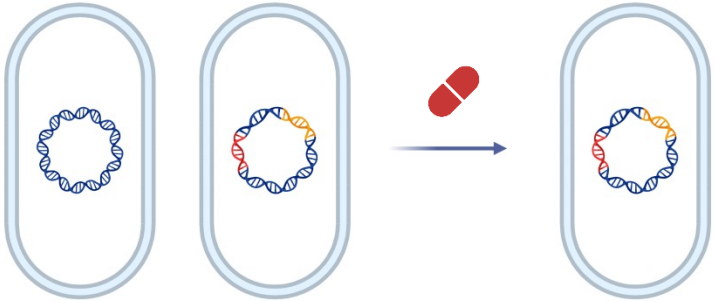


Antimicrobial 1 use induces resistance associated mutations (RAMs) to this antimicrobial

Indirect pathway



Antimicrobial 1 use selects for bacteria harboring RAMs to this antimicrobial



If RAMs associated to **antimicrobial 1** are frequently associated with RAMs to **antimicrobial 2**, then the use antimicrobial 1 will select for bacteria harboring RAMS to antimicrobial **1 and 2**

Methods

- We assessed the extent to which tetracycline RAMs were clonally distributed in *N. gonorrhoeae* and if these RAMS were associated with RAMs to other classes of antimicrobials
- Analysis of the 2375 gonococcal isolates from the 2018 Euro-GASP survey
  - Samples from individuals who had culture-positive gonococcal infection episodes in 26 European countries
  - Whole-genome sequencing was performed, and genogroups and AMR determinants were deduced
- RAMs for tetracycline: **rpsJ V57M, tetM**
- RAMs: gyrA, parC, penA, ponA, porB1a, mtr promoter, and folP.

Results

TABLE 1. Prevalence of Various Resistance Associated Mutations (RAMs*) According to Presence or Absence of *rpsJ* V57M and *tetM*

		rpsJ WT	*rpsJ* V57M	*tetM* Absent	*tetM* Present
gyrA RAMs* ↗	Absent	549 (47.5)	608 (52.6)	1001 (86.5)	156 (13.5)
	Present	10 (0.8)	1208 (99.2)†	854 (70.1)	364 (30.0)†
parC RAMs* ↗	Absent	553 (38.4)	886 (61.6)	1088 (75.6)	351 (24.4)
	Present	6 (0.6)	930 (99.4)†	767 (81.9)	169 (18.1)†
penA RAMs* ↗	Absent	177 (99.4)	1 (0.6)	176 (98.9)	2 (1.1)
	Present	382 (17.4)	1815 (82.6)†	1679 (76.4)	518 (23.6)†
folP RAM* ↗	Absent	303 (92.1)	26 (7.9)	321 (97.6)	8 (2.4)
	Present	256 (12.5)	1790 (87.5)†	1534 (75.0)	512 (25.0)†
mtrR promoter a57 del ↗	Absent	359 (20.3)	1406 (79.7)	1283 (72.7)	482 (27.3)
	Present	200 (32.8)	410 (67.2)†	572 (93.8)	38 (6.2)†
*ponA** ↗	Absent	373 (26.4)	1038 (73.6)	1029 (72.9)	382 (27.1)
	Present	186 (19.3)	778 (80.7)†	826 (85.7)	138 (14.3)†
*porB1a** ↗	Absent	555 (30.3)	1276 (69.7)	1345 (73.5)	486 (26.5)
	Present	4 (0.7)	540 (99.3)†	510 (93.8)	34 (6.3)†
mtrR promoter mosaic ↗	Absent	556 (26.0)	1581 (74.0)	1620 (75.8)	517 (24.2)
	Present	3 (1.3)	235 (98.7)†	235 (98.7)	3 (1.3)†
mtrD mosaic ✗	Absent	559 (26.0)	1589 (74.0)	1631 (75.9)	517 (24.1)
	Present	0 (0)	227 (100)	224 (98.7)	3 (1.3)†
tetM ↗	Absent	553 (29.8)	1302 (70.2)	NA	NA
	Present	6 (1.2)	514 (98.9)†	NA	NA

Number (row percentage).

**gyrA* (S91F, D95A, D95G, D95N), *parC* (D86N, S88P, E91K), *penA* (A311V, V316T, I312M, ins346D, T483S, P551S, G542S, G545S), *ponA* (L421P), *porB1a* (G120K, G120D/A121D), mtr promoter (a57del), and *folP* (R228S).

†$P < 0.001$.

NA indicates not applicable; WT, wild type.

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tetM	Absent	553 (29.8)	1302 (70.2)	NA	NA
	Present	6 (1.2)	514 (98.9)†	NA	NA

Number (row percentage).

**gyrA* (S91F, D95A, D95G, D95N), *parC* (D86N, S88P, E91K), *penA* (A311V, V316T, I312M, ins346D, T483S, P551S, G542S, G545S), *ponA* (L421P), *porB1a* (G120K, G120D/A121D), mtr promoter (a57del), and *folP* (R228S).

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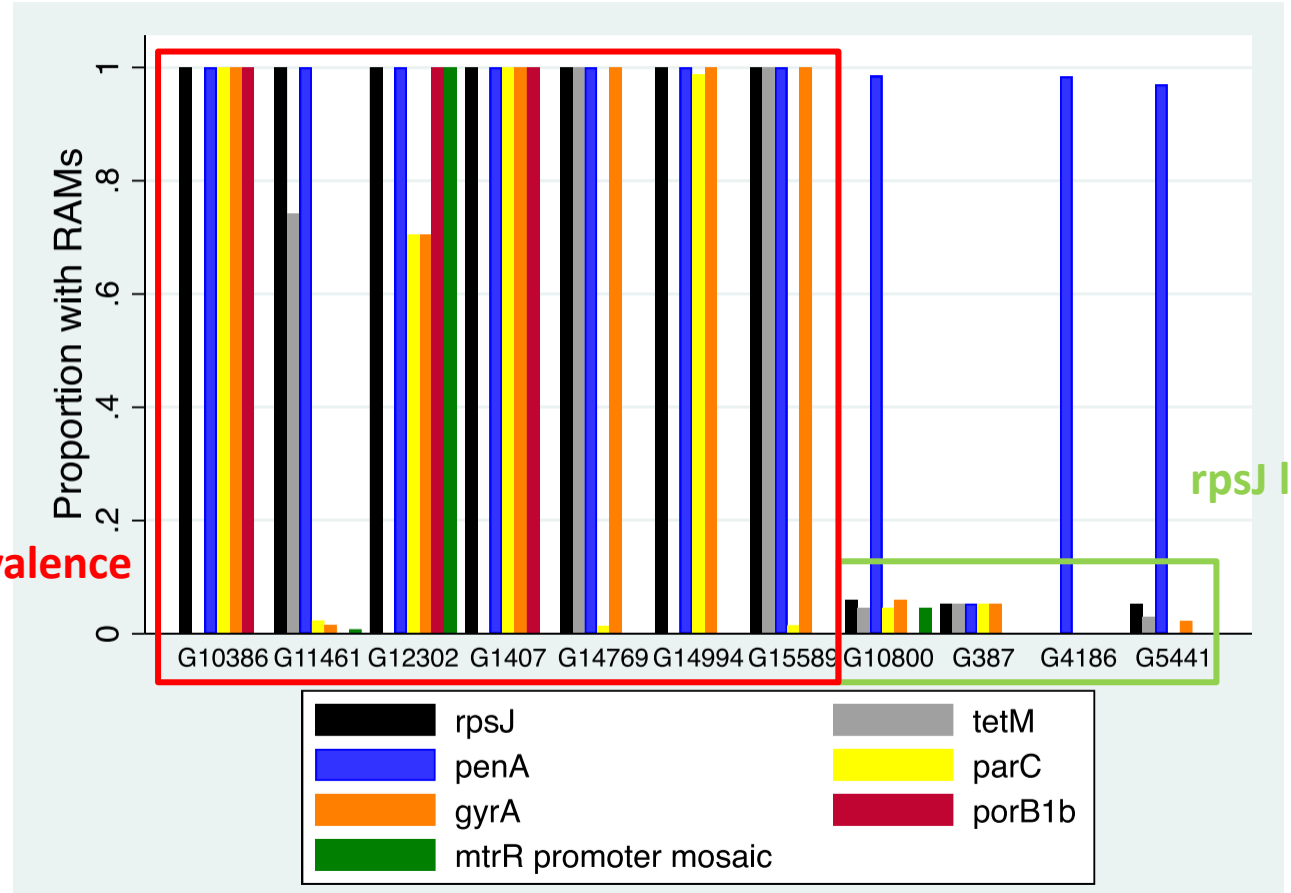
NA indicates not applicable; WT, wild type.

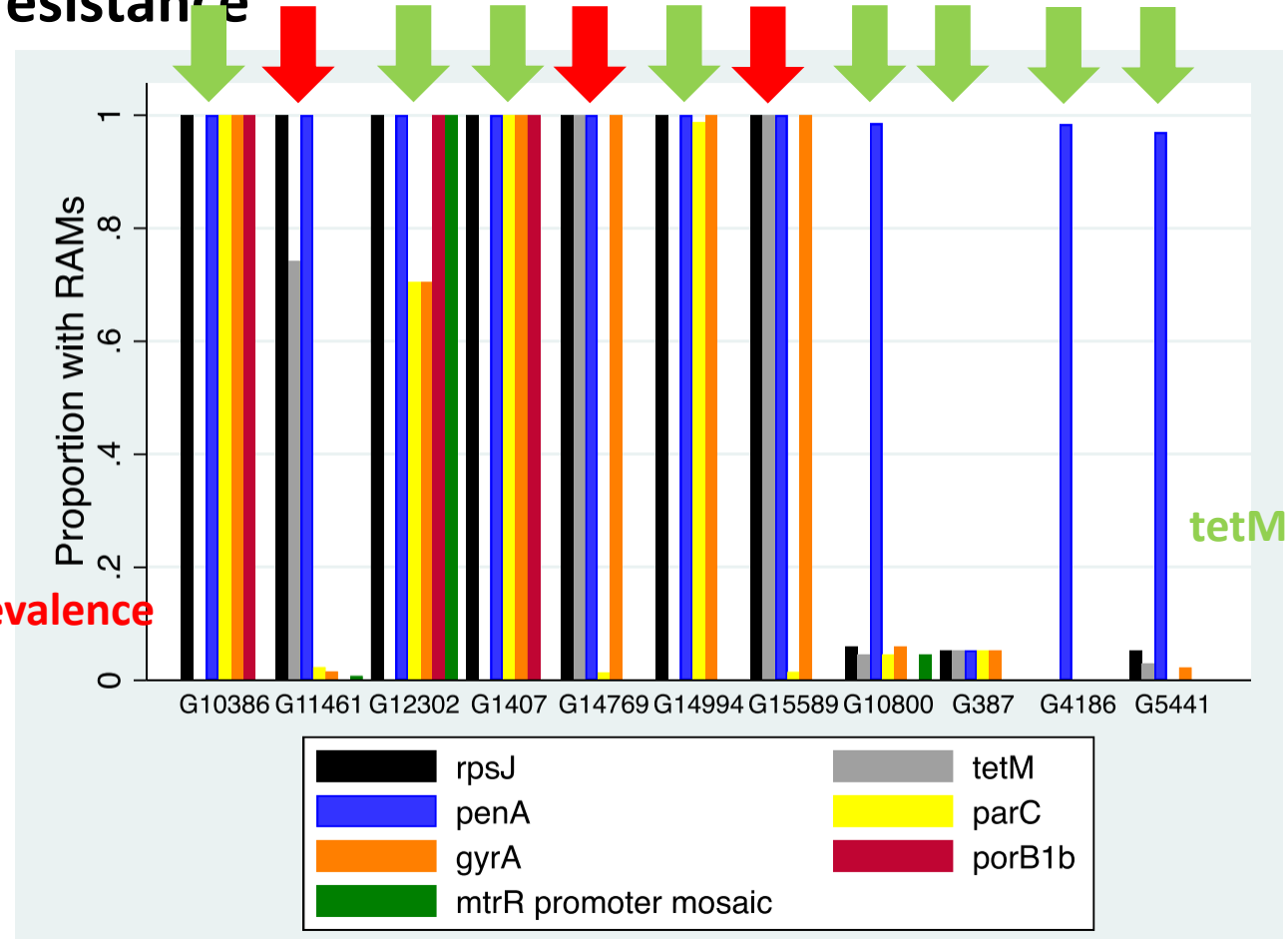
Cross resistance



rpsJ high prevalence

rpsJ low prevalence

Cross resistance



tetM high prevalence

tetM low prevalence

Conclusions

- We found strong individual- and genogroup-level associations between the presence of the **rpsJ V57M** mutation and **gyrA, penA, porB1a, mtrR promoter/mtrD, and folP** RAMs
- A number of the RAMs assessed were negatively associated with the presence of **tetM**
- Studies and programs using doxycycline PEP would be advised to monitor for the emergence of resistance to other antimicrobials in *N. gonorrhoeae* and other bacterial species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