Impact of Doxycycline as STI Postexposure Prophylaxis on the Gut Microbiome and Antimicrobial Resistance Gene Expression

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BACKGROUND

- Doxycycline post-exposure prophylaxis (doxy-PEP) use by MSM/TGW reduces incident bacterial STIs by 65%.
- Antimicrobial resistance is a global health concern. Rates of antimicrobial resistant infections are rising, including multidrug resistant *N. gonorrhoeae*.
- The impact of intermittent doxycycline exposure on the gut microbiome and on the associated antimicrobial resistance reservoir (resistome) is unknown.

METHODS

- **Study Design:** The DoxyPEP trial[1] was an open-label study with 501 participants (MSM/TGW with ≥1 bacterial STI in the last year) randomized 2:1 to doxy-PEP (200mg of doxycycline taken 24-72 hours of condomless sex, DP) and standard of care (SOC).
- **Laboratory Methods:** DNA and RNA metagenomic sequencing was performed on a subset of 150 participants (50 SOC, 100 DP) who had self-collected rectal swabs at enrollment (D0) and the 6-month visit (M6), which were frozen at -80°C. Specimens were selected from DP participants with highest reported doxy-PEP use. All samples which met minimum sequencing quality control were included in the analysis.
- **Statistical Analyses:** We compared gut bacterial microbiome (diversity and abundance) and resistome (antimicrobial resistance gene (ARG) abundance by DNA-seq; active ARG expression by RNA-seq) between SOC and DP participants and over time. P-values were calculated using Wilcoxon rank-sum test and adjusted for multiple comparisons.

RESULTS

GUT MICROBIOME

- Bacterial alpha diversity and beta diversity did not differ between SOC and DP arms at Day 0 or Month 6, or over time within each arm.
- Total bacterial abundance, normalized by reads per million sequenced, did not differ between SOC and DP arms at Day 0 or Month 6, or over time by arm.

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Doxy-PEP use minimally affected the gut microbiome over 6 months. **Increased expression of tetracycline antimicrobial resistance genes was observed,** *and future research is necessary to determine clinical significance.*

GUT RESISTOME

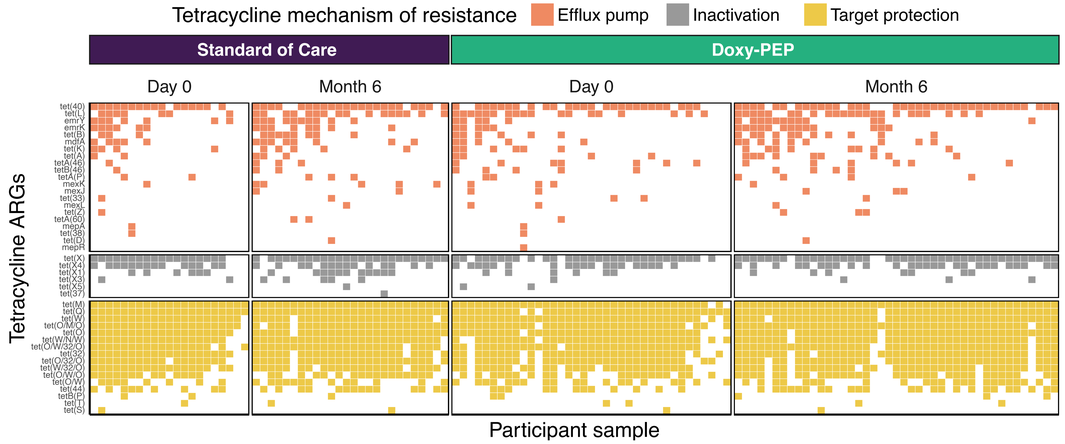


Figure 1. Heatmap of tetracycline ARGs by mechanism of action, visit, and study arm detected in the DNA-seq samples (n=127). Tetracycline ARGs were detected in all samples regardless of study arm or visit date. Ribosomal target protection was the most common mechanism of resistance.

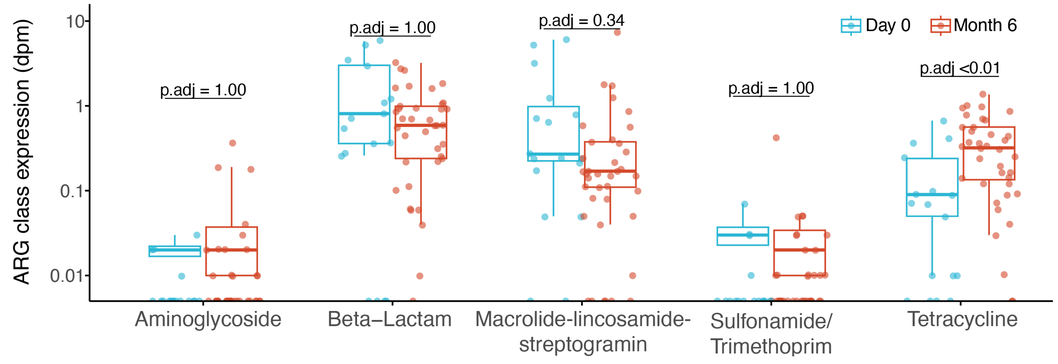


Figure 2. Impact of doxy-PEP use on ARG class expression, normalized by reads per million sequenced and gene length (depth per million, dpm) in the doxy-PEP RNA-seq samples (n=55). Tetracycline ARG expression significantly increased between Day 0 and Month 6, while no difference was observed among non-tetracycline ARG classes.

GUT RESISTOME

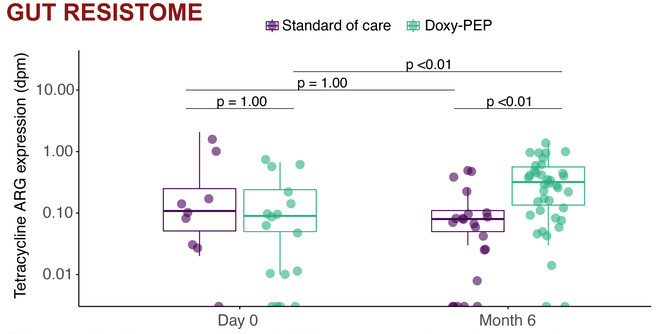


Figure 3. Tetracycline ARG expression by study arm and visit in the RNA-seq samples (n=86). Tetracycline ARG expression increased in the doxy-PEP Month 6 group compared with the SOC Month 6 and the doxy-PEP Day 0 groups.

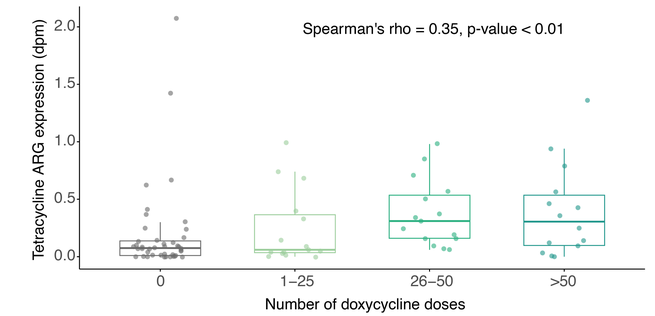


Figure 4. Tetracycline ARG expression increased with number of doxycycline doses since enrollment in the RNA-seq samples (n=86).

CONCLUSIONS

- Doxy-PEP use over 6 months did not significantly alter bacterial microbiome diversity or total bacterial abundance.
- Doxy-PEP use was associated with a dose-dependent increase in tetracycline ARG expression. The effects of longer use of doxy-PEP on the clinical significance of TCN ARG expression is a priority for future research.
- Active expression of non-tetracycline ARG classes was unchanged by doxy-PEP use.

REFERENCES

1. Luetkemeyer, A. F. *et al.* Postexposure Doxycycline to Prevent Bacterial Sexually Transmitted Infections. *New England Journal of Medicine* **388**, 1296-1306 (2023).

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