Antimicrobial Resistance (AMR) Consequences of the use of Doxycycline for Prevention of Bacterial Sexually Transmitted Infections (STIs): A Living Evidence Synthesis.

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BACKGROUND AND RATIONALE FOR THIS EVIDENCE SYNTHESIS

Rates of bacterial sexually transmitted infections (STI) such as gonorrhoea, syphilis, and chlamydia, have steadily increased in Canada in the past 20 years, disproportionately impacting some communities and groups, including gay, bisexual, and other men who have sex with men (GBMSM) (1–6). Between 2010 and 2019 there was a 33.1% increase in reported cases of chlamydia, reported cases of syphilis increased 393.1% and cases of gonorrhea increased 181.7% (7). Rates reduced during the COVID-19 pandemic due to less attention and therefore less demand for testing, and also more limited access to services related to sexually transmitted and blood-borne infections (STBBIs). In 2021, reported cases of Chlamydia from all over Canada was 104,426 cases for a rate of 273.2 case/100,000 population. There were 32,192 reported cases of gonorrhea for a rate of 84.2 cases/100,000 population and 11,540 cases of infectious syphilis were reported for a rate of 30.2 cases/100,000 population (8). Innovative and effective strategies to stop this rising trend in STI incidence are urgently sought. One strategy being researched is doxycycline prophylaxis as a STI prevention strategy (9–11). Antimicrobial resistance (AMR) is a concurrent public health issue in Canada, and some of the same groups and communities that are most affected by STI also bear a disproportionate burden of AMR, particularly AMR STI, including GBMSM. Stewardship of antibiotic use among these populations is an important consideration (12).

Doxycycline pre-exposure (Doxy-PrEP) and post-exposure prophylaxis (Doxy-PEP) have gained much interest as potential means of preventing STI in individuals and communities who are disproportionately affected and/or considered to be at high risk for STI, with published clinical studies focusing on cisgender GBMSM and transgender women (TGW), including individuals living with HIV and taking HIV PrEP (13,14).

Doxycycline, an antibiotic belonging to the tetracycline class, acts by stopping the synthesis of vital proteins, which in turn kills the bacteria (15). It is a recommended first-line treatment for chlamydia in Canada and the U.S (16,17) and an alternative treatment option for syphilis in Canada in non-pregnant individuals with penicillin allergy (18). Doxycycline is not a recommended treatment for gonorrhea, and background rates of gonorrhea resistance to tetracyclines are very high in Canada (64.6% in 2021) (19). Doxycycline use has been associated with the development of resistance to tetracycline antibiotics and with the development of resistance to other antibiotics for a range of bacteria (20–26)

There are currently no widely accepted guidelines for the use of Doxy-PrEP or Doxy-PEP, despite growing interest in these interventions among public health authorities, medical experts, and impacted populations. The San Francisco Department of Public Health and Seattle and King County Public Health (27,28) have published recommendations for Doxy-PEP prescribing for specific STIs and populations at highest risk of STI and the Australasian Society of HIV, Viral Hepatitis and Sexual Health Medicine (ASHM)

published recommendations for Doxy-PEP prescribing primarily for syphilis (13). The U.S centres for disease and control CDC posted a draft proposal for the use of Doxy-PEP for bacterial STI prevention in October 2023 (29) and the International Antiviral Society – USA Panel advises the use of Doxy-PEP for the prevention of bacterial STIs to be considered on a case-by-case basis (30). The U.K. Health Security Agency and the British Association for Sexual Health and HIV, do not support the use of Doxycycline for STI prophylaxis.

Given evidence that antibiotic use is associated with antimicrobial resistance at the individual, community, and population levels (31,32), a major concern with doxycycline prophylaxis is that its use may accelerate antibiotic resistance among bacterial STIs and other organisms, with disproportionate impacts for some populations (e.g. GBMSM) (11,33). In a 2022 systematic review, Truong et. al., reported that the use of oral tetracyclines for 2 – 18 weeks, may enhance antibiotic resistance in normal flora according to their analysis of data from small prospective studies (25). Statements and recommendations about Doxy-PEP/Doxy-PrEP consistently highlight the incompletely understood potential impacts of these interventions on the emergence and acceleration of antimicrobial resistance in chlamydia, gonorrhea and syphilis and other organisms.

A more thorough examination and synthesis of the data regarding the unintended consequences of antimicrobial resistance (AMR) resulting from the use of doxycycline in the prevention of bacterial STIs can aid PHAC in answering inquiries regarding the use of doxycycline in pre- and post-exposure prophylaxis (Doxy-PrEP/Doxy-PEP) for prevention of bacterial STIs as well as in making recommendations regarding its administration.

The aim of this review is to examine current evidence of the AMR consequences of the use of Doxy-PEP/Doxy-PrEP for the prevention of bacterial STIs.

REVIEW QUESTIONS.

Primary question: What are the possible antimicrobial resistance (AMR) consequences of the use of doxycycline for pre-exposure or post-exposure (Doxy-PrEP/Doxy-PEP) prophylaxis of bacterial sexually transmitted infections?

Secondary questions:

1. What is the effectiveness of Doxy-PEP/Doxy-PrEP for the prevention of bacterial STI at different population levels of tetracycline/doxycycline AMR?
2. What changes in tetracycline/doxycycline resistance have been observed in the context of Doxy-PEP/Doxy-PrEP **or** other prolonged or repeated doxycycline exposures, including the use of tetracyclines for the prevention or treatment of other infections (e.g. acne, malaria)? (Note: it is important to specify the method for measuring and reporting tetracycline/doxycycline resistance e.g. using MIC distribution method, proportion exceeding defined resistance thresholds, resistance-associated mutations, or strain selection)

3. What is known about the emergence of antimicrobial cross-resistance, including multi-drug resistance, in the context of Doxy-PEP/Doxy-PrEP use **or** other prolonged or repeated doxycycline exposures, including the use of tetracyclines for the prevention of other infections (e.g. acne, malaria)?
4. Through modelling studies that account for different levels of baseline resistance and expected efficacy, what are the predicted changes in tetracycline/ doxycycline resistance.

RESEARCH METHODS:

The methods outlined below have all been defined a priori and documented in the study protocol.

Literature Search

Search strategy:

A literature search for studies published between 2013 and 2023 was conducted on January 4, 2024, on PubMed, Embase, Cochrane via OVID and pre-print servers (MedRxiv). The reference lists of the included studies were also searched for other relevant studies, but none were identified. Appendix 1 shows the detailed search strategy.

Eligibility Criteria

Population/Jurisdictions: Global. Studies including any population (adults and children) – with an expected focus in the published literature on key populations of interest including those who experience disproportionate burdens of both STI and AMR (e.g. GBMSM and TGW, including people living with HIV and people taking HIV PrEP).

Study designs: Eligible studies include Randomized controlled trials (RCTs) including cluster RCTs, cohort studies, case-control, ecological studies and systematic reviews (published or unpublished, including abstracts and conference proceedings). Case reports and case series were not eligible.

Interventions/Indicators (Exposure): Prolonged or recurrent use of doxycycline of any dose and for any indication that include but are not limited to Doxy-PEP, Doxy-PrEP, prevention of malaria, treatment of acne, suppression of prosthetic joint infection, and leptospirosis prophylaxis.

Comparators/Controls: non-tetracycline class of antibiotics, no treatment, non antibiotics, or standard of care.

Outcomes of interest:

- Effectiveness of PrEP and PEP for gonorrhea, chlamydia (including lymphogranuloma venereum, LGV) and syphilis disaggregated by population rate of tetracycline or doxycycline resistance.
- Baseline and follow-up rate of tetracycline-resistance among target organisms (*N. gonorrhoeae).*
- Baseline and follow-up rate of tetracycline-resistance among non-target organisms [as available: commensal *Neisseria, M. genitalium*, *E. coli*, *Shigella spp*., *Campylobacter, S. aureus, S. pyogenes, S. pneumoniae, K. pneumoniae, Rickettsia* species, other spirochetes (e.g. *Borrellia burgdorferi*), *Vibrio* species, *Leptospira, Yersinia* species, *Francisella tularensis, Brucella* species, *Bacillis*

anthracis, *Plasmodium* species, *Mycoplasma* species, *Mycobacterium marinum, Chlamydia* and *Chlamydophila* species, and methicillin-resistance *S. aureus*]

- Baseline and follow-up rate of *N. gonorrhoeae, C. trachomatis* and *T. pallidum* resistance to other antimicrobials (as available: penicillins, cephalosporins, macrolides, fluoroquinolones)
- Baseline and follow-up rate of non-target organism resistance to other antimicrobials, including *M. genitalium* resistance to other antimicrobials (as available: macrolides and fluoroquinolones) and rates of AMR in sexually transmissible enteric infections (STEI), e.g. AMR *Shigella spp*.

RESULTS

What we found

From the database searches, a total of 4767 studies were identified and imported onto Covidence. After the removal of 85 duplicates, 4682 titles and abstracts were screened. The first 20% were screened by two reviewers (MU and CK), one reviewer (MU) then screened the remaining 80%, while the second reviewer (CK) screened all excluded studies. After the title and abstract screening, 125 studies were retrieved and assessed for eligibility at the full text stage. A first reviewer screened all 125 studies, and the second reviewer screened all excluded studies. All conflicts were resolved through discussions. Reasons for excluding any source of evidence at this stage were noted. After excluding a further 108 studies at this stage, a total of 17 studies made it to the data extraction stage. Following directions in the study protocol, the first reviewer extracted relevant data from the 17 studies while the second reviewer checked for correctness and completeness of the extracted data. See figure 1 for the Prisma flow diagram detailing the screening process.

Of the 17 studies, there were two systematic reviews (25,34), nine studies from eight randomized controlled trials (35–44) - (two sub-studies (39,40) were obtained from one randomized controlled trial, i.e. the ANRS IPERGAY trial), three observational cohort studies (45–47), two analytical cross sectional studies (48,49), and one modelling study (50).

Of the 17 studies, no study was found that addressed the 1st research question about the effectiveness of Doxy-PEP/Doxy-PrEP for the prevention of bacterial STI at different population levels of tetracycline/doxycycline AMR. Twelve studies (25,34,48,49,51,35–38,42,43,45,46) addressed different aspects of the 2nd research question on observed changes in tetracycline/doxycycline resistance in the context of Doxy-PEP/Doxy-PrEP **or** other prolonged or repeated doxycycline exposures, including the use of tetracyclines for the prevention or treatment of other infections (e.g. acne, malaria). Three studies (25,46,51) addressed aspects of the 3rd research question - What is known about the emergence of antimicrobial cross-resistance, including multi-drug resistance, in the context of Doxy-PEP/Doxy-PrEP use **or** other prolonged or repeated doxycycline exposures, including the use of tetracyclines for the prevention of other infections (e.g. acne, malaria)? And one study (50) was found that addressed some parts of the 4th research question – findings in modelling studies around the impact of doxycycline and other tetracyclines on AMR. See table 1 for details of included studies.

Figure 1: Prisma flow diagram

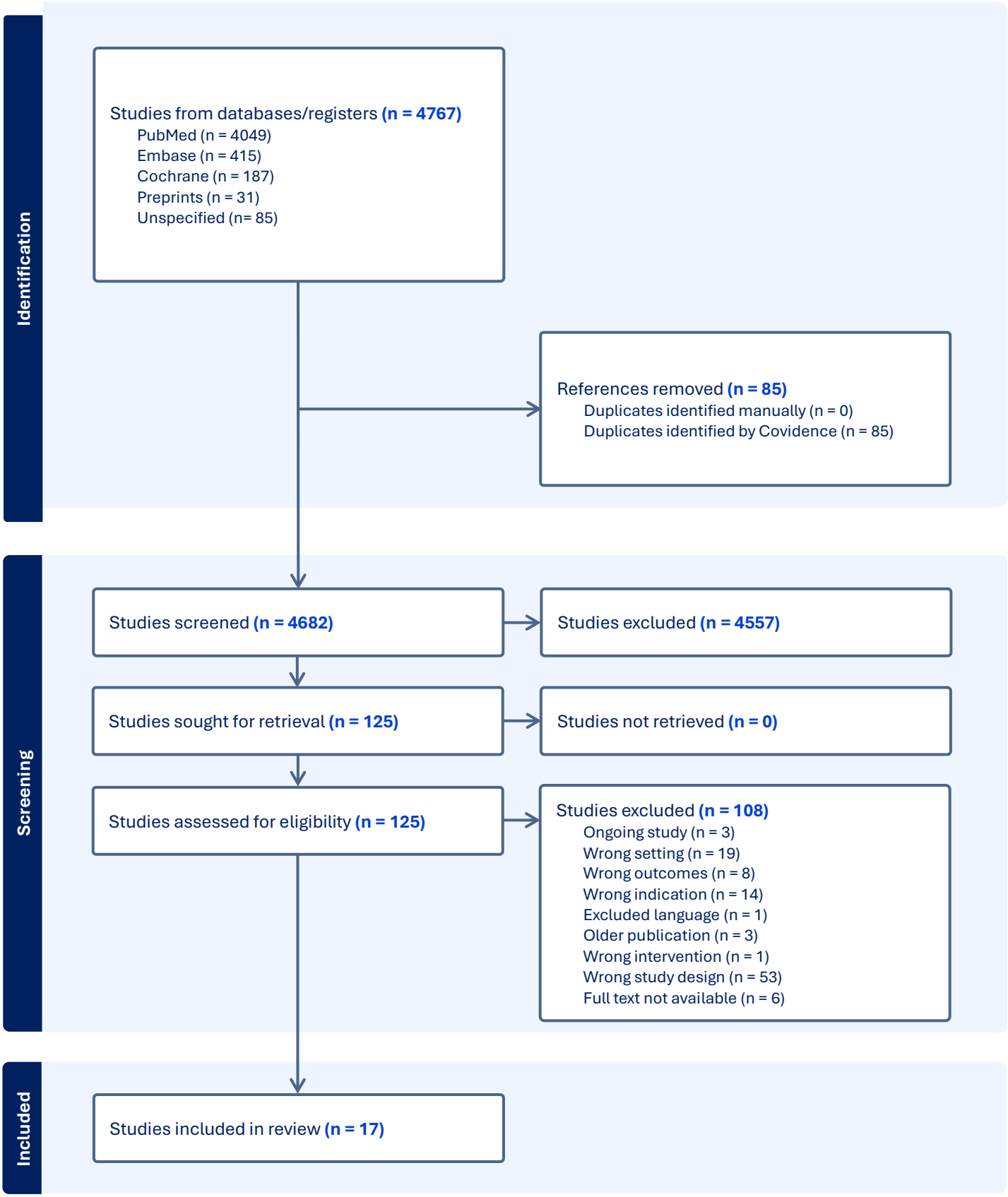

Table 1: Characteristics of included studies.

Study ID and status	Design	Population Inclusion criteria	Analysis	Type of Treatment/Prophylaxis	Comparator	AMR consequences considered
Alkhawaja et al., 2020, Jordan (51) Published	Cross-sectional	Acne outpatients using systemic or topical antibiotics for the treatment of acne	Chi-square and t-test tests.	Antibiotics. Systemic - Doxycycline Topical – Clindamycin and Erythromycin	Non-users	Antibiotic resistance of *Cutibacterium acnes* towards used antibiotics
Jo et. al., 2021, USA. (35) Published	Randomized single-center, longitudinal, interventional pilot study.	Healthy adults able to comply with antibiotic administration, microbiome sampling procedures, and longitudinal follow-up after ingestion of antibiotics for up to 1 year. Randomized into 4 groups with at least 3 participants per group.	Wilcoxon rank-sum test. "paired = T" parameter	**3 Antibiotic classes:** 1. Doxycycline 20 mg or Doxycycline 100 mg 2. Cephalexin 500mg 3. Trimethoprim/sulfamethoxazole **4 standard oral regimens:** 1. Doxycycline 20 mg twice daily for 56 days 2. Doxycycline 100 mg twice daily for 56 days 3. Cephalexin 500mg 3 times daily for 14 days, 4. Trimethoprim/sulfamethoxazole 160/800mg twice daily for 14 days	Untreated	The selection, expansion, and persistence of antibiotic-resistant strains (mostly Staphylococci, in particular *S. epidermidis* and *S. hominis*) on skin both during and after systemic antibiotic use.
Kantele et. al., 2022, Finland (46) Published	**A 2-part study:** 1. Prospective cohort study **2.** Literature review	Travel history to low- and middle-income country (LMIC)	Pearson's chi-square test, Fisher's exact test Binary logistic regression analysis	Doxycycline usage	Non-users	Prospective cohort study portion examined the impact of doxycycline usage on 1. Acquisition of extended spectrum beta lactamase-producing Enterobacterales (ESBL-PE)

						2. Doxycycline co-resistance among travel-acquired ESBL-PE isolates in in relation to doxycycline use The literature review portion examined level of doxycycline/tetracycline resistance on 1. ESBL-PE 2. Stool pathogens
Luetkemeyer et. al., 2023, USA (36,37) Published (36) Unpublished – conference proceedings (37)	Randomized open-label trial.	Men who have sex with men (MSM) and transgender women (TGW) living with HIV or on PrEP with history of *N. gonorrhoeae*, *C. trachomatis*, and syphilis in the past year. Randomized 2:1	ITT Fisher's exact test	200 mg of doxycycline within 72 hours after condomless sex	Standard care	Effect of Doxy-PEP use on antimicrobial resistance (AMR) in *N. gonorrhoeae S. aureus* and Neisseria spp. Tetracycline resistance in *N. gonorrhoeae* and *S. aureus* isolated at baseline as compared with organisms isolated during study follow-up.
Mende et. al., 2016, USA (45) Published	Observational cohort study.	Active-duty personnel or department of Defense (DoD) beneficiaries, who sustained deployment-related injuries requiring medical evacuation through Landstuhl	Chi-square, Fisher's exact test (Fisher–Freeman-Halton test with Monte Carlo as appropriate), and Mann–Whitney U test.	Doxycycline exposure - defined as rseeceipt of the antibiotic after medical evacuation and prior to isolate collection	No doxycycline exposure	Characteristics related to tetracycline resistance, including doxycycline exposure, in *S.. aureus* isolates.

		Regional Medical Center (LRMC)				
Nakase et. al., 2022, Japan (49) Published	Observational cohort study	Patients with acne	Fisher's exact test	Previous Antibiotic use for acne	No previous treatment	The impact of antimicrobial treatment of acne vulgaris on skin bacteria (*S. epidermidis*) antimicrobial resistance.
Reichert and Grad, 2023, USA. (50) Preprint	Modelling study	The model characterized an US MSM population (N = 10^6) stratified into 3 sexual activity groups characterized by annual rates of partner change.	Deterministic compartmental model transforming the model into a susceptible-exposed-infectious-susceptible (SEIS)model R package deSolve to observe the projected dynamics of ceftriaxone and doxycycline resistance, as well as the burden of gonococcal infection, following DoxyPEP implementation at t = 0.	DoxyPEP uptake	No uptake	A mathematical model to investigate the impact of DoxyPEP for gonorrhea prevention on **resistance dynamics** and the burden of infection in men who have sex with men (MSM).
Sermswan et. al., 2023, Thailand (48) Published	Cross-sectional	Patients with mild to severe facial Acne vulgaris (AV) and have received Treatment medication within	$\chi 2$ and Fisher exact test	Antibiotic usage (including tetracycline and doxycycline)	Not used	Prevalence of antibiotic-resistant *C. acnes* and its association with acne severity with use of topical and systemic antibiotic treatments.

		the previous 3 months.				
Teles et. al., 2021, USA (38) Published.	Single-center, randomized, laboratory-blind, parallel, two-arm controlled clinical study	Patients with Stage III and Stage IV periodontitis Had received active periodontal treatment within 1 year before enrollment. Aged >25 years In good general health. Had at least four sites with PD >4 mm.	Mean percentage Mann-Whitney test Friedman test.	Supra- and sub-gingival debridement done with minocycline microspheres.	Debridement done without minocycline microspheres	Impact of minocycline hydrochloride microspheres on the shifts of oral bacterial species (e.g. *Gemella morbillorum, Eubacterium saburreum, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, and Porphyromonas gingivalis)* resistant to minocycline.
Vanbaelen et. al., 2024, (26) Published.	Systematic Review and Meta-Analysis	RCTs comparing the efficacy of PEP tetracycline with either placebo or no treatment for reducing the incidence of bacterial STIs and reporting the prevalence of tetracycline resistance in any bacterial species at baseline and study end.	Mann-Whitney test to compare tetracycline MIC distribution of isolates per species of the two arms post PEP. A random-effect model was used to combine the results for the meta-analysis	Tetracyclines at any dosage	Placebo or no treatment	Antimicrobial resistance (AMR) to tetracyclines in all bacterial *(N. gonorrhoeae, S. aureus and commensal Neisseria species)* species with available data
Truong et. al., 2022, Canada (25)	Systematic review	RCTs that compared the impact of daily	Descriptive report due to much	Oral tetracycline class antibiotics	Non-tetracycline control	The impact of oral tetracycline-class

Published		oral tetracycline class antibiotics vs a non-tetracycline control on the acquisition of tetracycline class AMR in normal flora among adults.	variability in outcomes of included studies.		(placebo, no antibiotic use or alternative oral antibiotics)	antibiotics on AMR in normal flora.
Bercot et. al., 2021, France (39) Published Molina et al., 2018, France (40) Published Both are sub studies of the same RCT	Randomized open-label trial.	Asymptomatic MSMS enrolled in the open-label phase of the ANRS IPERGAY trial and using PrEP for HIV. Randomized 1:1	ITT. Chi-square or Fisher's exact tests used for significance of differences in percentages as appropriate.	Doxycycline 200 mg within 24 hours after each sexual intercourse (with a limit of 600 mg/week)	No prophylaxis	To screen *Mycoplasma genitalium* isolates for mutations associated with resistance to macrolides, fluoroquinolones and **tetracyclines (doxycycline).** Doxycycline resistance in *N. gonorrhoeae*
Brill et. al 2015, UK (42)	Exploratory 13-week, single-centre, single-blind, placebo controlled, randomised controlled trial.	Stable patients aged ≥45 years with COPD, FEV_1<80% predicted and chronic productive cough. Randomized 1:1:1:1	ITT A linear mixed effects model for log(MIC) for multiple isolates within each individual was used to model antibiotic resistance. A generalised mixed effects model was used to analyse resistance as a binary outcome.	**3 antibiotic regimens for 13 weeks** 1. Moxifloxacin 400 mg daily for 5 days every 4 weeks 2. Doxycycline 100 mg/day 3. Azithromycin 250 mg 3 times a week	One placebo tablet daily for 13 weeks	Antimicrobial resistance in airways bacteria in all treatment arms. Changes in resistance to the three tested antibiotics

Grennan et. al., 2021. Canada (43) Unpublished – conference proceedings	Randomized controlled open-label pilot study	HIV negative MSM or TGW with history of syphilis Randomized 1:1	ITT Fishers Exact test to compare STI rates between those on dual PrEP vs. HIV PrEP alone over the initial 24.	Immediate daily doxycycline 100mg for 48 weeks	Deferred doxycycline beginning 24 weeks	Tetracycline/doxycycline resistance in *S. aureus*
Stewart et al., 2023, Kenya (44) Published	Randomized, open-label trial	Non pregnant women on HIV PrEP. Randomized 1:1	ITT	200mg Doxycycline within 24-72 hours after sex	Standard of care	Doxycycline resistance in *N. gonorrhoeae*
Molina et. al., 2024, France (41) Published.	Randomized, open-label trial	MSM taking PrEP against HIV with a bacterial STI within the prior 12 months. Randomized 2:1 to DoxyPEP or no PEP and 1:1 to 4CMenB vaccination or no vaccine.	ITT	200mg Doxycycline within 24-72 hours after sex Note: This report only covers the DoxyPEP or no PEP arms.	No PEP	Doxycycline resistance in *N. gonorrhoeae* and *C. trachomatis*

Evidence Appraisal

Risk of bias:

One reviewer assessed the risk of bias for each outcome in the included studies and the second reviewer verified all judgements and support statements. The Cochrane Risk of Bias tool version 2.0 (RoB 2.0) (52) was used to assess the risk of bias (RoB) for seven RCTs (eight studies) of the eight included RCTs or nine studies (see figure 2). RoB was classified as "some concerns" in seven studies (36,38–42,44) and “high risk” in one (35). A RoB assessment was not completed for one RCTs (43) due to the unavailability of the full text; data retrieved originated from a conference proceedings. The ROBINS-E tool (54) was used for the assessment of RoB in the three observational studies. RoB was classified as "some concern" in one (49). Serious risk for potential confounding was identified in two studies (45,46) and as such further assessment was not needed. See Table 2 for the RoB assessment for the observational cohort studies. The Joanna Briggs Institute (JBI) Critical Appraisal Checklist for analytical cross-sectional studies (55) was used to critically appraise the two cross sectional studies.(48,51). See Table 3 for a detailed critical appraisal of the included cross-sectional studies. The AMSTAR 2 tool for assessing the methodological quality of systematic reviews (56) was used to critically appraise the two included systematic reviews (25,34). Vanbaelen et. al., was judged to be a critically low quality review while Truong et. al., was considered to be a low quality review. See appendices 2a and 2b for details. Due to the unavailability of an established quality assessment tool for mathematical modeling studies, a quality assessment was not conducted for the mathematical modeling study (50).

A meta-analysis of the included studies was not undertaken due to the lack of comparability in terms of design, population, interventions, and comparators across all studies reporting the same end point.

Certainty of Evidence.

Using the GRADE system (57), the first reviewer assessed the degree of certainty in the evidence within the GRADE domains of risk of bias assessment, inconsistency, indirectness, imprecision of effect estimates and publication bias. The second reviewer verified all judgements and rationales. The GRADE assessment was done for outcomes where multiple original studies were available and a well-established tool for a risk of bias assessment was available.

Certainty of evidence was completed for studies addressing the second secondary research question on observed changes in tetracycline/doxycycline resistance in the context of Doxy-PEP/Doxy-PrEP **or** other prolonged or repeated doxycycline exposures, including the use of tetracyclines for the prevention or treatment of other infections i.e. sub-question 2a - Changes in tetracycline/doxycycline resistance for *N. gonorrhoeae* observed in the context of prophylactic doxycycline use; sub-question 2b - Baseline rates of tetracycline/ doxycycline resistance for *M. genitalium* and observed changes in the context of prophylactic doxycycline use or other prolonged or repeated doxycycline exposures; and sub-question 2c - **C**hanges in tetracycline/doxycycline resistance observed for non-target organisms in the context of doxycycline use.

Certainty of evidence was also completed for studies addressing the third secondary research question on what is known about the emergence of antimicrobial cross-resistance, including multi-drug resistance, in the context of Doxy-PEP/Doxy-PrEP use or other prolonged or repeated doxycycline exposures, including the use of tetracyclines for the prevention of other infections, specifically sub-question 3b - Impact of prophylactic doxycycline use on levels of resistance to other antimicrobials for non-target organisms. The GRADE summary of findings table (Table 4) is shown below.

Fig 2: Risk of bias assessment for included RCTs

Table 2: Risk of bias assessment for included observational cohort studies (ROBINS-E Tool) for the outcome of tetracycline/ doxycycline resistance among non-target organism.

Author, year. Ref.	Confounding	Exposure measurement error	Selection bias	Post exposure interventions	Missing data	Outcome measurement Error	Selective reporting	Overall judgement
Nakase et. al., 2022. (49)	Some concerns	Low	Some concerns	Low	Some concerns	Low	Low	Some concerns
Kantele et. al., 2022.[a] (46)	Serious	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated	High risk
Mende et. al., 2016.[a] (45)	Serious	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated	High risk

Abbreviation: ROBINS-E, Risk of Bias in Non-randomized Studies—of Exposure.

[a] Further assessment was not necessary as the risk for potential confounding was deemed serious in the first domain.

Table 3: Critical appraisals for included analytical cross-sectional studies.

Reference	Alkhawaja et. Al., 2020				Sermswan et. Al., 2023			
	Yes	No	Unclear	Not applicable	Yes	No	Unclear	Not applicable
Were the criteria for inclusion in the sample clearly defined?	☑	□	□	□	☑	□	□	□
Were the study subjects and the setting described in detail	☑	□	□	□	☑	□	□	□
Was the exposure measured in a valid and reliable way?	☑	□	□	□	☑	□	□	□
Were objective, standard criteria used for measurement of the condition?	☑	□	□	□	☑	□	□	□
Were confounding factors identified?	□	□	☑	□	□	□	☑	□
Were strategies to deal with confounding factors stated?	□	□	☑	□	□	□	☑	□
Were the outcomes measured in a valid and reliable way?	☑	□	□	□	☑	□	□	□
Was appropriate statistical analysis used?	☑	□	□	□	☑	□	□	□
Overall Appraisal	Include ☑		Exclude □	Seek further information. □	Include ☑		Exclude □	Seek further information. □

Table 4: GRADE summary of findings

Number of studies and design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Upgrade consideration	Quality
Question: Changes in tetracycline/doxycycline resistance for *N. gonorrhoeae* observed in the context of prophylactic doxycycline use. **Setting:** People taking PrEP for HIV and people living with HIV. **Reference:** Luetkemeyer 2023; Molina 2018; Stewart 2023, Molina 2024							
4 RCTs[a]	No serious limitations	Very serious limitations (-1)	No serious limitations	Very serious limitations (-1)	Undetected	None	⊕⊕⊕◯ Moderate
Question: Baseline rates of tetracycline/ doxycycline resistance for *M. genitalium* and observed changes in the context of prophylactic doxycycline use or other prolonged or repeated doxycycline exposures. **Setting:** People taking PrEP for HIV **Reference:** Bercot, 2021							
1 RCT	No serious limitations	Serious limitations	Serious limitations	Very serious limitations (-1)	Undetected	None	⊕⊕⊕◯ Moderate
Question: Changes in tetracycline/doxycycline resistance observed for non-target organisms in the context of doxycycline use. **Setting:** Diverse[b] **Reference:** Jo 2021; Teles 2021; Luetkemeyer 2023; Brill 2015 (RCTs) and Kantele 2022; Nakase 2022; Mende 2016 (Cohort)							
4 RCTs[a]	No serious limitations	Very serious limitations (-1)	Very serious limitations (-1)	Very serious limitations (-1)	Undetected	None	⊕⊕◯◯[d] Low
3 Cohorts	Very serious limitations[c]	Not serious limitations	Serious limitations	Serious limitations	Undetected	None	⊕◯◯◯[e] Very low
Question: Impact of prophylactic doxycycline use on levels of resistance to other antimicrobials for non-target organisms. **Setting:** Persons with travel history to low- and middle-income country (LMIC). **Reference:** Kantele 2022							
1 Cohort	Very serious limitations	Not serious limitations	Serious limitations	Serious limitations	Undetected	None	⊕◯◯◯[e] Very low

[a] GRADE assessment not done on some included RCTs because of the unavailability of full texts.
[b] The studies are very different in terms design, population, interventions, and comparators and outcome measures, there was therefore no consistency.

[c] Two studies considered to be at very high risk of bias.
[d] Level of evidence is low; new evidence will likely influence the findings.
[e] Level of evidence is very low, it is therefore likely that new evidence will influence the findings.

KEY FINDINGS:

Four secondary research questions were formulated to help answer the Primary research question which is "What are the possible antimicrobial resistance (AMR) consequences of the use of doxycycline for pre-exposure or post-exposure (Doxy-PrEP/Doxy-PEP) prophylaxis of bacterial sexually transmitted infections?

Secondary research question 1*:*

What is the effectiveness of Doxy-PEP/Doxy-PrEP for the prevention of bacterial STI at different population levels of tetracycline/doxycycline AMR?

a) At different population levels of *N. gonorrhoea* resistance to tetracycline/doxycycline, what is the effectiveness of Doxy-PEP/Doxy-PrEP for its prevention, disaggregated by sex, subpopulation, and/or anatomical site of infection (i.e. pharynx, urethra, cervix, rectum)?

We did not find any study addressing this research question.

Secondary research question 2:

What changes in tetracycline/doxycycline resistance have been observed in the context of Doxy-PEP/Doxy-PrEP or other prolonged or repeated doxycycline exposures, including the use of tetracyclines for the prevention or treatment of other infections (e.g. acne, malaria)?

a) What changes in tetracycline/doxycycline resistance for *N. gonorrhoeae* have been observed in the context of prophylactic doxycycline use?

Five studies, four RCTs (36,40,41,44) and one systematic review (34) addressed this question. All the studies detected an increase in tetracycline resistance in *N. gonorrhoeae* when comparing baseline data with follow-up data.

The first study by Luetkemeyer et. al., 2023, was a randomized open-label trial among men who have sex with men (MSM) and transgender women (TGW) living with HIV or on PrEP with history of *N. gonorrhoeae*, *C. trachomatis*, and syphilis in the past year. They tried to understand the effect of doxy-PEP use on AMR of *N. gonorrhoeae*, *S. aureus*, and *Neisseria* spp. Participants were randomized 2:1 to 200 mg doxycycline within 72 hours of condomless sex or no doxycycline and observed for 12 months. Cultures for the different organisms were obtained at the beginning of the study – month 0 (M0) and at the end of the study – month 12 (M12). Among participants with *N. gonorrhoeae*, 17% (44/256) had phenotypic susceptibility results; Month 0 (M0), tetracycline resistance was detected in 27% (4/15) of *N. gonorrhoeae isolates*. After enrollment, tetracycline resistance was observed in 38.5% (5/13) of the isolates in the doxy-PEP arm and 12.5% (2/16) of the isolates in the no doxy arm, demonstrating no statistically significant difference in *N. gonorhoeae* resistance to tetracycline between the two arms (36). Vanbaelen et. al., 2024 calculated confidence intervals (CI) of 0.68 to 27.98 for this study in their meta-analysis(34). The wide CIs show how the study's power was limited by the small number of isolates (58). The authors warned that Doxy-PEP might not provide much protection against incident tetracycline resistance in *N. gonorrhoea* and

advocated for the necessity to monitor both, the effects of tetracycline resistant gonococci on doxy-PEP efficacy, and also the impact of doxy-PEP on gonococci resistance (36,37). Similarly, Hazra et al. concluded that the ability of Doxy-PEP to promote resistance to tetracyclines and other antibiotics should be assessed through larger population based trials (58).

In an open-label randomized study conducted between July 2015 and January 2016, Molina et. al., 2018 aimed to assess the effectiveness of post-exposure prophylaxis (PEP) with doxycycline in reducing the incidence of bacterial STIs among HIV-negative MSM or TGW who were on HIV PrEP. Participants were assigned 1:1 to take 200 mg doxycycline up to 72 hours after condomless sex or no prophylaxis. In addition to the primary end point of the occurrence of a first STI (gonorrhoea, chlamydia, or syphilis) during the follow-up period, the authors also assessed doxycycline resistance in *N. gonorrhoeae and C. trachomatis.* At enrolment and every 2 months after, participants were tested for syphilis, chlamydia and gonorrhoeal infections using anal and throat swabs and first-void urine samples. Whenever it was possible, cultures were also tested for gonorrhoea and chlamydia (40).

Tetracycline resistance was defined as a MIC > 1 mg/L and intermediate resistance was MIC > 0·5 mg/L but ≤1 mg/L. Tetracycline resistance was confirmed by molecular detection by PCR to test for acquisition of the tetM gene which is associated with high levels of tetracycline resistance and the Val57Met mutation in the rpsJ gene. In addition, they also assessed the overexpression of the MtrCDE-encoded efflux pump by screening for mutations in the promoter of the mtrR gene or in the MtrR protein. Genotyping and cell cultures to determine tetracycline MICs in vitro were conducted on all positive PCR samples for *C trachomatis* from throat and anal swabs (40).

A positive culture for gonorrhoea was obtained from 8 participants (2 in the PEP arm and 6 in the no-PEP arm) from 9 of 28 samples (32%) cultured for gonorrhoea. Tetracycline resistance was detected in 4 and intermediate resistance in 3 *N gonorrhoeae* isolates. All isolates from the no PEP arm were fully resistant. The tetM gene was detected in one of the resistant isolates. The Val57Met mutation in the rpsJ gene was carried by all resistant strains as well as mutations associated with overexpression of the antibiotic efflux pump MtrCDE (40).

Due to the very small sample size (limited amount of *N. gonorrhoeae* and *C. trachomatis* isolates available for antibiotic sensitivity testing) and short follow up period, the authors were not able to assess the effect of Doxy-PEP on the selection and dissemination of antibiotic resistance for STIs. They therefore proposed for studies that will assess the full effect of this strategy on the selection of antibiotic resistance for bacterial STIs and advised that until such data are available, the use of doxycycline as PEP should be restricted to research purposes (40). The authors did not distinguish between baseline and follow up data and data was not disaggregated anatomically, even though specimens were obtained from various anatomic sites.

Between February 5, 2020, and October 30, 2022, Stewart et. al., 2023 conducted a randomized, open-label trial which compared doxycycline PEP (200 mg doxycycline taken within 72 hours after condomless sex) vs standard care among non-pregnant adult Kenyan women on HIV PrEP. The authors compared tetracycline resistance *in N. gonorrhea* and *C. trachomatis* between the study arms in addition to the primary outcome of any incident infection with *C. trachomatis*, *N. gonorrhoeae*, or *Treponema pallidum*.

A total of 449 participants were randomized 1:1 (224 to the doxycycline-PEP group and 225 to the standard-care group) and were followed quarterly over 12 months. STI testing and treatment were done quarterly.

Endocervical swabs obtained from participants positive for *N. gonorrhoeae* at a follow-up visit were purified, and the bacterial DNA was extracted. The extracted DNA samples were tested for the tetracycline resistance gene tet(M) of the American- and Dutch-type plasmids, by PCR. Tet(M) gene confers high-level tetracycline-resistant *N. gonorrhoeae*. Specimens for genotypic resistance testing were collected in 16 enrollment visits at baseline. All (100%) were positive for tet(M) gene. This was also the case for the follow-up visits with all (100%) of the DNA samples obtained in 20 visits from the doxycycline-PEP group and in 12 visits from the standard-care group being positive for tet(M) gene (44).

The most recent trial, the ANRS 174 DOXYVAC study by Molina et. al., 2024, is a 2 x 2 factorial randomized, open-label trial among MSM on HIV PrEP for over 6 months who have had a bacterial STI in the past 12 months, with no STI symptoms upon enrolment. A total of 720 MSM were randomized 2:1 to Doxy-PEP (200 mg within 72 hours of condomless sex) or no PEP and 1:1 to 2 shots of 4CMenB vaccination or no vaccination, to determine superiority of DoxyPEP or 4CMenB over no doxycycline or no vaccination. Median follow-up time was 9 months (interquartile range 6 – 12) with quarterly visits. At baseline and every three months and when symptomatic, participants were tested for *N. gonorhoeae*, *C. trachomatis* and *M. genitalium* by PCR from 3 sites (throat, anus and urine) while serology testing was done for syphilis every 3 months (41,59). There were 78 gonorrhoea cultures available for testing for tetracycline susceptibility. All isolates were resistant at baseline and at follow up. Of 31 isolates in the Doxy-PEP arm, 11 (36%) and 5 of 40 (13%) isolates in the no doxy arm had high level resistance ($p = 0.043$). Resistance was determined using E-test and EUCAST 2023 breakpoints (Resistance: MIC > 0.5 mg/L; high level resistance: MIC > 8 mg/L) (41).

In their systematic review, Vanbaelen et. al., 2024 (26), searched for RCTs reporting the prevalence of tetracycline resistance in any bacterial species at baseline and trial end, and comparing the effectiveness of tetracycline with either placebo or no therapy for lowering the incidence of bacterial STIs. For *N. gonorrhoea*, they found that regardless of whether resistance was defined as ≥ 1 or ≥ 2 mg/L, there was no statistically significant difference in the prevalence of tetracycline resistance in *N. gonorrhoeae* between the tetracycline and placebo arms in any of the three trials included in the study. Neither was there any significant difference in the pooled estimates (OR 2.3; 95% CI 0.9-5.9).

Of the three RCTS included in the Vanbaelen SR, two (36,40) met criteria for this review and their results have been summarized above. The third RCT (60) was excluded as it was outside of the date range. This systematic review concluded that *N. gonorrhoeae* tetracycline MICs were considerably higher in the minocycline arm (median 2 mg/L IQR 1.5-2.5 mg/L) than in the placebo arm (median MIC 1.5 mg/L, IQR 1-2 mg/L; $p = 0.0018$). While 10/44 (22.7%) of the isolates in the placebo arm had MICs in the range of < 1.5mg/L, none of the gonococcal isolates in the minocycline arm had a MICs of less than 1.5 mg/L. The authors explained that the difference observed in the 2 outcome measures [by proportion (≥ 1 or ≥ 2 mg/L) vs MIC distribution] could be due to the fact that changes in MIC distribution provide a more sensitive estimate of the effectiveness of an antibiotic than the proportion of resistant isolates when MIC distribution is dichotomized into resistant and susceptible (34). Their study concluded, PEP with tetracyclines like minocycline and doxycycline could be associated with the selection of tetracycline resistance in *N. gonorrhoeae.*

No tetracycline resistance was observed with *C. trachomatis* in the 3 studies that provided such data (40,41,44). In the Molina 2018 study, 5 samples (21% of 22 anal and 2 oral swabs) from 4 participants (2 in each arm) yielded a positive culture for chlamydia. All tetracycline MICs were within normal range (0·12–0·25 mg/L) (40). Stewart et. al. had a total of 76 *C. trachomatis* DNA samples collected for tet(C) gene testing; 20 at baseline and 56 at follow up (25 in the doxy-PEP arm and 31 in the standard of care arm). The tet(C) gene cassette was detected in none (0%) (44). Finally, in the Molina 2024 study, 4 strains (all from the no PEP arm) were tested for tetracycline resistance in culture; there was no resistance observed. Likewise, no tetracycline-resistant mutation was detected in the 16S rRNA of 68/126 (54%) of *C. trachomatis* PCR positive swabs sequenced. Eight sequences were from the Doxy-PEP arm (41)

Details of studies addressing baseline and follow-up rate of tetracycline-resistance among target organisms are shown in Table 2.

b) <u>What baseline rates of tetracycline/ doxycycline resistance for *M. genitalium* exist, and what changes have been observed in the context of prophylactic doxycycline use or other prolonged or repeated doxycycline exposures?</u>

This question was addressed by one RCT (39) and one systematic review (34).

In their study Bercot et. al., 2021 detected in vivo mutations of *M. genitalium* 16S rRNA associated with tetracycline resistance in other bacteria in 2 of 16 (12.5%) specimens tested; corresponding to an overall rate of *M. genitalium* 16S rRNA mutations of 14.3% (2/14; 95% CI, 1.8–42.8%). There were no minimum MIC data to confirm any doxycycline resistance.

Detected mutations were at positions C1192G, G966T, and C967T within or very close to the tetracycline target site. All these resistant strains were detected in participants with history of doxycycline use. Hence, the use of doxycycline may have facilitated the acquisition of these mutations. The authors therefore concluded that the potential tetracycline resistance associated mutations observed in their study raise important issues for the screening and treatment of *M. genitalium* in asymptomatic individuals, thereby supporting recommendations to avoid testing or treatment of asymptomatic *M. genitalium* infection (39).

The above study was included in the Vanbaelen et. al., 2024, systematic review. Commenting on tetracycline/ doxycycline resistance in *M. genitalium*, they remarked that the study analyzed a very small sample size for 16s rRNA mutations (n = 11 at baseline and n = 5 at the 6 month follow up). There was no statistically significant difference in the prevalence of suspected tetracycline resistance between the tetracycline (1/2) and no tetracycline arms (0/3) at 6 months (34). See table 3 for details.

c) <u>What changes in tetracycline/doxycycline resistance have been observed for non-target organisms in the context of doxycycline use? i.e. How does prophylactic doxycycline use or other prolonged or repeated doxycycline exposures affect tetracycline/doxycycline resistance levels for non-target organisms?</u>

- Non-target organisms noted in the Doxy-PEP/Doxy-PrEP literature include commensal *Neisseria, M. genitalium*, *E. coli*, *Shigella spp*., *Campylobacter, S. aureus, S. pyogenes, S. pneumoniae, K. pneumoniae*. Other non-target organisms of interest include *Rickettsia* species, other spirochetes

(e.g. *Borrelia burgdorferi*), *Leptospira*, *Vibrio* species, *Yersinia* species, *Francisella tularensis, Brucella* species, *Bacillus anthracis*, *Plasmodium* species, *Mycoplasma* species, *Chlamydia* and *Chlamydophila* species, and some methicillin-resistance *S. aureus*.

Twelve studies, 5 RCTs (35,37,38,42,43), two systematic reviews (25,34), three observational cohort studies (45,46,49) and two cross-sectional studies (48,51) addressed this question. Of the twelve studies that addressed this question, a GRADE assessment was completed for four RCTs (35,36,38,42) and three observational cohort studies (45,46,49) Due to variations in study participants, interventions and comparators, there were very serious limitations in inconsistency, indirectness and imprecision and as such, certainty of evidence was rated low for the RCTs and very low for the cohort studies.

GRADE assessment was not completed on one RCT (43) because there is no full text available, two systematic reviews (25,34) and two cross-sectional studies (48,51).

- ***Randomized controlled trials:***

Three of the RCTs (35,37,38) were conducted in the US, one in the UK (42) and one in Canada (43). They were all very different in terms of design, population, interventions, comparators, and outcomes.

The study of Jo et. al., 2021 was a pilot RCT which looked at the effect of different classes of commonly prescribed antibiotic regimens for dermatological disorders on short- and long-term alterations of the skin microbiome. One of the study aims was to look at the selection, expansion, and persistence of antibiotic-resistant strains on skin both during and after systemic antibiotic use. Healthy adults were randomized into 4 groups with at least 3 participants per group. Each group was administered one of four standard oral regimens (Doxycycline 20 mg twice daily for 56 days, Doxycycline 100 mg twice daily for 56 days, Cephalexin 500mg 3 times daily for 14 days or Trimethoprim/sulfamethoxazole 160/800mg twice daily for 14 days). Patients were followed for up to one year. Data analysis of the skin microbiome of untreated healthy individuals, also followed for up to one year (short intervals of 1 to 2 months: n= 10; and long interval of > 1 year: n ≥ 5) were used for comparison. Swabs were obtained from three skin sites (antecubital crease, retroauricular crease and the volar forearm) with different physiological and microbiological characteristics before, during and after antibiotic use (≥6 time points per participant). Antibiotic resistance was investigated by culturing and sequencing bacterial isolates from pre and post antibiotic skin swabs. Findings from this study demonstrated that antibiotic-resistant bacteria are selected for, expand, and remain on skin during and after systemic antibiotic usage. The study also demonstrated that antibiotics can disrupt the homeostasis of skin microbiota and can cause significant alterations in the resistome, some of which can last almost a year after stopping the antibiotics (35). Their findings in relation to emergence and selective expansion of doxycycline-resistance are enumerated below. Doxycycline resistant strains (mostly Staphylococci, in particular *S. epidermidis* and *S. hominis*) were only isolated after participants were given doxycycline, suggesting that the antibiotic acted as a selective pressure for these resistant strains.

- Skin swabs were collected and cultured on days 0, 14, 56, 112 and 336. No resistant colonies was isolated on day 0 (pre-antibiotic). Resistant strains were isolated on days 14, 56, 112 and 336.
- The plasmid-associated tetK or tetL genes, which encode efflux pumps known to confer resistance to tetracyclines, were present in all doxycycline-resistant *S. epidermidis* isolates (MIC ≥ 2 μg/ml).
- Additionally, the MIC values of doxycycline-resistant isolates from Doxy100 subjects were higher than those from Doxy20 subjects.

- Possibly due to a longer treatment duration (56 days), Doxy100 subjects were observed to show significant and persistent changes in their skin microbiota that lasted longer than 200 days, whereas the skin microbiomes of subjects on other antibiotic regimens of 14 days duration returned to a baseline state after Day 42.
- The predominant doxycycline-resistant strains varied among subjects at each body site, which is consistent with variations in the microbial communities found in anatomically distinct skin sites. This strongly suggests that skin site characteristics also play a role in antibiotic-driven selection.
- The abundances of tetK and tetL genes evaluated in each individual and site similarly supported this site-dependent observation.

The study of Luetkemeyer et. al., 2023, a randomized open-label trial (36,37), sought to look at the effect of Doxy-PEP use on AMR in *N. gonorrhoeae, S. aureus* and *Neisseria* spp among MSM and TGW living with HIV or on PrEP with a history of *N. gonorrhoeae*, *C. trachomatis*, and syphilis in the past year. The participants were randomized 2:1 to 200 mg doxycycline within 72 hours of condomless sex and standard of care with no doxycycline. The participants were followed for 12 months.

At baseline, *S. aureus* was found in 139 of 326 participants in the Doxy-PEP arm, with 12 (8.6%) being Doxy-R. In the SOC arm, *S. aureus* was found in 76 of 157 participants, with 13 (17.1%) being Doxy-R. MSSA was present in 118 of 326 participants in the Doxy-PEP arm, with 11 (9.3%) being Doxy-R, and in 67 of 157 participants in the SOC arm, with 9 (13.4%) being Doxy-R. MRSA was isolated from 20 participants in the Doxy-PEP arm, with 1 (5%) being Doxy-R, and from 9 participants in the SOC arm, with 4 (44.4%) being Doxy-R.

At month 12 (M12), *S. aureus* was found in 31 of 111 participants in the Doxy-PEP arm, with 5 (16.1%) being Doxy-R. In the SOC arm, *S. aureus* was found in 24 of 51 participants, with 2 (8.3%) being Doxy-R. MSSA was present in 29 of 111 participants in the Doxy-PEP arm, with 5 (17.2%) being Doxy-R, and in 21 of 51 participants in the SOC arm, with 1 (4.8%) being Doxy-R. MRSA was isolated from 2 participants in the Doxy-PEP arm, with 0 (0%) being Doxy-R, and from 3 participants in the SOC arm, with 1 (33.3%) being Doxy-R. The only statistically significant difference at 12 months was the lower rate of *S. aureus* carriage in the Doxy-PEP arm, while the higher proportion of doxycycline resistance in the Doxy-PEP arm was not statistically significant.

From the same unpublished data (37), the authors also reported their findings for commensal *Neisseria spp*. At M0, *Neisseria spp* were cultured from 86.8% of participants, with doxy-R *Neisseria* detected in 61.8% of them (37). At baseline, 266/302 (88.1%) had *Neisseria spp* with 62.6% (189/302) of them being Doxy-R in the doxy-PEP arm vs 129/153 (84.3%) with doxy-R in 60.1% (92/153) in the no doxy arm. At 12 months, *Neisseria* spp were cultured from 85.2% (104/122) in the doxy-PEP arm and 89.3% (50/56) in the no doxy arm ($p = 0.64$); doxy-R was 69.7% (85/122) in the doxy-PEP arm and 44.6% (25/56) in the no doxy arm ($p=0.017$).

In summary, the authors claimed that *S. aureus* colonisation was decreased by 16% by doxy-PEP without a significant increase in doxy-R *S. aureus*. At baseline, most individuals had commensal doxy-R *Neisseria*, with a surprising decline in doxy-R *Neisseria* spp. in the SOC arm (37) The authors concluded that the little variations in doxy-R *S. aureus* and *Neisseria* spp. are probably not clinically significant, and they need to be seen in the context of doxy-PEP's >60% STI reduction (37) and advised that further investigations and longer follow-up will be required to ascertain how Doxy-PEP maybe linked to significant selection of

resistance in commensal oropharyngeal Neisseria spp, other STIs like *M. genitalium* and gut microbiome (36)

In the RCT of Teles et. al., 2021, the use of minocycline microspheres resulted in a transient selection of minocycline resistant species in saliva and subgingival plaque samples. The study, a single-center, randomized, laboratory-blind, parallel, two-arm controlled clinical study looked at the impact of minocycline hydrochloride microspheres on the shifts of oral bacterial species resistant to minocycline in patients with stage III and stage IV periodontitis. One arm had supra- and sub-gingival debridement done with minocycline microspheres while debridement was done without minocycline microspheres in the other arm. Participants were followed for 6 months and clinically monitored with the collection of saliva and subgingival plaque samples at baseline, 1, 3, and 6 months. The test group's saliva and plaque samples showed an initial increase in the mean percentage of resistant isolates after one month and then a decrease at six months ($P < 0.05$). However, the control group showed no change.

In an exploratory trial in stable patients with COPD, Brill et. al., 2015 compared three antibiotic regimens (moxifloxacin 400 mg daily for 5 days every 4 weeks, doxycycline 100 mg/day and azithromycin 250 mg 3 times a week) against placebo (one placebo tablet daily) for 13 weeks in order to assess and compare their effects on total airway bacterial load in stable COPD as well as to evaluate antimicrobial resistance (changes in resistance to the tested antibiotics) in airways bacteria in the treatment arms (42). The authors observed measurable increases in the degree of antibiotic resistance of isolates in all treatment arms. Baseline MIC and whether the isolate could be linked with lower respiratory tract infections were adjusted for, and doxycycline was observed to be associated with a factor increase of 3·74 (95% CI 1.46 to 9.58, p=0.01) compared with placebo. Where MIC breakpoints were available, isolates from participants in the doxycycline arm were found to be more likely to be resistant to doxycycline than those from participants in the placebo arm (OR 5.77 (95% CI 1.40 to 23.74, p=0.02)). The authors concluded that the observed large increases in antibiotic resistance could have major consequences for future studies (42).

Though unpublished and therefore not yet peer-reviewed, Grennan et. al., 2021, in an open-label pilot study to determine STI outcomes of HIV-negative MSM or TGW on dual HIV/STI PrEP, also assessed tetracycline resistance in *S. aureus* isolates obtained from the nares of study participants. Participants (n =52) were randomized 1:1 to receive either immediate daily doxycycline 100mg (n = 26) for 48 weeks, or deferred doxycycline beginning at 24 weeks later (n = 26). Tetracycline resistance was determined by Kirby-Bauer disk diffusion susceptibility test. In the immediate arm, tetracycline resistance was observed in 1 of 3 *S. aureus* isolates at 24 weeks and in 3 of 6 isolates at 48 weeks, while in the deferred arm, resistance was seen in 1 of 2 *S. aureus* isolates at 48 weeks. Although the numbers were small, the authors recommended re-examining this demonstration of the development of tetracycline resistance in commensal organisms in larger studies (43).

Systematic reviews:

The systematic review of Vanbaelen et. al., 2024 also included the study of Luetkemeyer et. al., 2023, the results of which have been summarized above (36). In summarizing the findings, Vanbaelen et. al. remarked that the prevalence of tetracycline resistance in commensal *Neisseria* species was higher in the tetracycline PEP arm vs the placebo arm in the only study they identified where this was assessed (OR 2.9, 95% CI 1.5-5.4). And for *S. aureus*, there was no statistically significant difference in the prevalence of tetracycline resistance between the tetracycline and placebo arms in the only study where this was assessed (OR 2.1; 95% CI 0.4-12.0) (26).

Truong et al., looked at the changes in AMR measures (resistance genes, MIC and/or susceptibility) from baseline to follow-up between the intervention and comparator groups, per tested bacterial species and antibiotic in their systematic review of the impacts of oral tetracycline class antibiotics on antimicrobial resistance in normal human flora. Data were mostly from small prospective studies with intervention arms receiving varying types and doses of the tetracycline class of antibiotics (Doxycycline 100 -200mg/day = 5 studies; Tetracycline 1000mg/day = 1 study; Oxytetracycline 1000 mg/day and Minocycline 100 mg/day (in different intervention groups) = 1 study) and they compared with equally varying comparators (Placebo = 3 studies; Non-antibiotic controls = 3 studies; Combination of placebo and alternative antibiotics = 1 study). Intervention period ranged from 2 -18 weeks. There was evidence of differing baseline tetracycline resistance for both the intervention and comparison arms in all included studies. Results from the studies also differed, but most reported an increased burden of tetracycline resistance. The authors concluded that based on limited data from small prospective studies, oral tetracyclines for 2–18 weeks may promote resistance in subgingival, gastrointestinal, and upper respiratory tract flora, despite the effects being mild and temporary and suggested that AMR in commensal bacteria should be included in STI prophylactic trials (25).

- ***Observational cohort studies:***

Kantele et. al., 2022 conducted a 2-part study, a prospective cohort study and a literature review. The study participants were a cohort of 412 Finnish travellers to low- and middle- income countries. Doxycycline 100 mg daily was used as malaria prophylaxis among the travellers. The authors assessed the impact of doxycycline use on traveller's diarrhea rates as well as its impact on doxycycline resistance among stool pathogens. All four RCTs reviewed in the literature review portion of their study, reported on enterotoxigenic *Escherichia coli* (ETEC) or various *E. coli* isolates with higher doxycycline/tetracycline resistance rates among doxycycline users than non-users when assessing the impact of doxycycline use on doxycycline/tetracycline resistance rates among various stool bacteria (46). In another study they reviewed, which compared doxycycline users to mefloquine users, tetracycline resistance was found in 77% vs 35% of all ETEC strain and 100% vs 50% of all *Campylobacter* strains among doxycycline users vs mefloquine users respectively. All studies were done among US military based in different regions (46).

Nakase et. al., 2022, in their study with patients with acne in Japan found that the proportion of *S. epidermidis* strains with the resistance genes tet(M) was significantly higher in patients who had used tetracycline vs those who did not use any antimicrobial (p <0.05), suggesting that the use of antimicrobials for acne treatment may lead to an increased prevalence of antimicrobial-resistant *S. epidermidis*, thereby advocating for appropriate antibiotic use in acne treatment. On the other hand, no difference was observed in the prevalence rate of strains with tet(K) gene (49). They also correlated the presence of resistance genes with phenotypic resistance: 44 of 46 (95.7%) of strains with tet(M) gene were resistant to minocycline but susceptible to doxycycline, whereas 20 of 53 (37.7%) of strains with tet(K) gene were resistant to doxycycline when using CLSI breakpoints, but susceptible to minocycline. All strains without tet(M) or tet(K) genes were susceptible to both doxycycline and minocycline. The study demonstrated that the use of tetracyclines for acne treatment led to a notable increase in the prevalence of minocycline resistant *S. epidermidis* with tet(M) genes and the acquisition of tet(M) and tet(K) genes by *S. epidermidis* differs by resistance levels to tetracyclines (49)

Mende, et. al., 2016 examined the impact of doxycycline antimalarial prophylaxis on *S. aureus* tetracycline resistance among military personnel injured and repatriated to the US from either Iraq or Afghanistan.

Doxycycline exposure was defined as receipt of the antibiotic after medical evacuation and prior to isolate collection.

Doxycycline exposure: A total of 168 *S. aureus* isolates were analyzed. Of these, 92 isolates were from patients exposed to doxycycline. Doxycycline was administered a median of 2 days post-injury (IQR: 2–3 days) and isolate recovery was a median of 50 days (IQR: 2–3 days) post injury. There was no significant difference regarding methicillin and tetracycline resistance and pulse field type (PFT) profiles between the isolates from doxycycline-exposed vs unexposed patients. Overall, the tet genes profile was not significantly different between doxycycline-exposed vs unexposed patients; however, doxycycline-exposed patients had a statistically higher proportion of tet(M) genes, which are associated with tetracycline resistance, than non-exposed patients (P = 0.031).

Tetracycline class resistance: Of the 168 *S. aureus* isolates analyzed, 45 (27%) were MRSA and 38 (23%) were resistant to antimicrobials in the tetracycline class. Among the 38 (23%) isolates resistant to tetracyclines, all of them 38/38 (100%) were resistant to tetracycline, 25/38 (66%) were resistant to doxycycline and 23/ 38 (61%) were resistant to minocycline. Among the tetracycline resistant isolates, tet(M) and tet(K) genes were the most prevalent tetracycline resistance genes.

Among the 25 isolates resistant to doxycycline, 17 (68%) were from patients with antimalarial prophylaxis. There was no significant difference in tetracycline resistance between isolates obtained from patients who received antimalarial prophylaxis and those that did not (45).

- ***Cross-sectional studies:***

In a cross-sectional study of 155 patients with acne and presenting to selected dermatology outpatient clinics in Jordan, Alkhawaja et. al., 2020 sought to assess the prevalence and acquisition of antibiotic resistance to antibiotics in *C. acnes* and other Gram-positive skin flora. They analyzed the antibiotic resistance patterns of *C. acnes* clinical isolates and that of *S. aureus* and *S. epidermidis* isolated from the skin in association with the use of any antibiotic (systemic or topical). Sixty-0ne (61%) of the patients had reported prior treatment with antibiotics and 39 (39%) did not. The antibiotic used by patients were systemic doxycycline and topical clindamycin and erythromycin. Thirty-seven (37%) of the *C. acne* isolates were resistant to Doxycycline, 36% were resistant to Tetracycline while 3% were resistant to minocycline. Of the *S. aureus* isolates 47% of the *S. aureus* isolates were resistant to Doxycycline 33% were resistant to Tetracycline while 8% were resistant to minocycline. And for *S.. epidermidis* isolates, 57% were resistant to Doxycycline, 48% were resistant to Tetracycline while 24% were resistant to minocycline. However, there was no significant difference in the antibiotic resistance profile between the two groups (antibiotic therapy vs no antibiotic therapy); however, a numerically higher number of resistant isolates was observed in the antibiotic therapy group (51)

In another cross-sectional study, Sermswan et. al., 2023 sought to find the association between prevalence of antibiotic resistance and history of any oral antibiotic usage among patients with acne in Thailand. Although, doxycycline was noted to be the most common antibiotic used, the authors do not specify which antibiotic was used. Tests for antibiotic susceptibility to trimethoprim/sulfamethoxazole, clindamycin, erythromycin, tetracycline, and doxycycline was performed.

The authors did not find any statistical difference between the prevalence of antibiotic resistance, including MDR strains, and a history of oral antibiotics (p= 0.823) or topical antibiotics (p= 0.464), although, there was a high prevalence of AMR and MDR. Doxycycline resistance was observed in 73 of 143 (51%) *C. acnes* isolates (48).

Details of studies addressing baseline and follow-up rate of tetracycline-resistance among non-target organisms can be found on Table 4.

- ***Summary:***

In summary, the evidence on sub-question 2c, while showing some mixed results, suggests a potential sizeable impact on resistance patterns in some non-targeted bacteria from prolonged exposure to tetracyclines, e.g. for Staphylococci spp including *S. aureus,* but also in *Neisseria spp* and in *E. coli* including ETEC. Some studies, however, have suggested no significant impact on the antibiotic resistance profiles, though, that include commensals such as *C. acne, S. aureus* and *S. epidermidis* isolates.

Secondary research question 3:

What is known about the emergence of antimicrobial cross-resistance, including multi-drug resistance, in the context of Doxy-PEP/Doxy-PrEP use or other prolonged or repeated doxycycline exposures, including the use of tetracyclines for the prevention of other infections.

a) What impacts of doxycycline use on *N. gonorrhoeae, C. trachomatis, and T. pallidum* resistance to other antimicrobials have been observed?

We did not find any study addressing this research question. However in a recent review discussing some areas of concerns regarding the widespread use of Doxy-PEP, Kong et.al., 2023 reported additional data from Luetkemeyer's unpublished data (37) that we have not found elsewhere. These data report some acquisition of cross-resistance to other antibiotics in *N. gonorrhoeae*. In comparison to isolates from the SOC arm, *N. gonorrhoeae* from participants in the Doxy-PEP arm apparently showed resistance to a higher number of other antibiotics. Three isolates from among 20 Doxy-PEP users were found to be resistant to azithromycin, two to ciprofloxacin and one to benzylpenicillin. Whereas among 19 participants in the SOC arm, only two *N. gonorrhoeae* isolates showed additional resistance to benzylpenicillin. Although the small sample sizes make it difficult to conclude statistical significance, there was no evidence of resistance to the two antibiotics currently used to treat gonorrhoea, ceftriaxone and cefixime (61).

b) What impacts of doxycycline use on non-target organisms have been observed? i.e. How does prophylactic doxycycline use affect levels of resistance to other antimicrobials for non-target organisms (i.e. cross resistance)? In considering cross resistance, we were particularly interested in understanding impacts on non-target multi-drug resistant organisms with a special focus on multi-drug-resistant (MDR) *M. genitalium* and multi-drug-resistant (MDR) / extensively drug resistant (XDR) *Shigella spp*.

Three studies were found that addressed this question -

Both the prospective cohort study and the literature review portions of the study of Kantele et. al.,2022, looked at the impact of doxycycline use on the acquisition of extended spectrum beta lactamase producing Enterobacterales (ESBL-PE). The cohort study did not identify any significant differences in the ESBL-PE acquisition rates between doxycycline users vs non-users (11/46 (23.9%) versus 79/ 366 (21.6%); p = 0.719), and findings from the literature review portion suggested neither an increased nor a decreased risk of contracting ESBL-PE among doxycycline users versus non-users (46).

The impact of doxycycline use on co-resistance among ESBL isolates to other antimicrobials did not differ significantly between doxycycline users and non-users (46): resistance to Ciprofloxacin [8/11 (72.7%) vs 40/79 (50.6%); p = 0.169]; tobramycin [6/11 (65.5%) vs 41/79 (51.9%); p = 0.869]; nitrofurantoin [0/11 (0%) vs 2/79 (2.5%); p = 0.594], and to co-trimoxazole [9/11 (81.8%) vs 57/79 (72.2%; p = 0.497].

In this study, doxycycline did not seem to enhance ESBL-PE acquisition despite the high rate of doxycycline co-resistance among the isolates. The authors explained that this was probably due to the high (> 80%) background rate of doxycycline resistance among the ESBL-PE isolates. The sample size was not large enough to detect that kind of small difference, but it was sufficient to detect selective pressure in the co-resistance rates. Doxycycline resistant ESBL-PE was found in 100% of doxycycline users and 82.3% of non-users (46).

In the 2016 study by Mende et. al., the authors didn't find any statistically significant difference in the overall resistance profile to other antibiotics between the doxycycline exposed and non-exposed groups. However, they observed that a significantly higher proportion of isolates from doxycycline exposed patients were resistant to levofloxacin and moxifloxacin than those from non-exposed patients (25% vs 10%; P = 0.016) for both antibiotics (45)

One of the secondary outcomes for the Truong et. al., 2022 systematic review was changes in resistance to non-tetracycline antibiotics (i.e. resistance genes, MIC and/or susceptibility) from baseline to follow-up between the intervention and comparator groups, per tested bacterial species and antibiotic (25). The impact of oral tetracyclines on outcomes pertaining to resistance to non-tetracycline antibiotics was assessed in 3 of the 7 included studies. Altogether, the findings of these studies showed that oral tetracyclines had very little effect on non-tetracycline resistance in commensal *E. coli* in the gastrointestinal system and *Propionibacterium* (*Cutibacterium*) species obtained from skin swabs. In comparison with no intervention, a study conducted among Peace Corps volunteers revealed a brief rise in various resistant commensal and pathogenic *E. coli* in stool isolates following three weeks of doxycycline treatment; this was followed by a return to baseline two weeks post treatment (25).

See table 5 for details of studies baseline and follow-up rate of non-target organism resistance to other antimicrobials, including *M. genitalium* resistance to other antimicrobials**.**

Secondary research question 4:

Through modelling studies that account for different levels of baseline resistance and expected efficacy, what are the predicted changes in tetracycline/ doxycycline resistance.

a) What changes in tetracycline/doxycycline resistance for *N. gonorrhoeae* have been modelled in the context of Doxy-PEP/PrEP use or other prolonged or repeated doxycycline exposures to

doxycycline, including the use of tetracyclines for the prevention or treatment of other infections (e.g., acne, malaria)?

One modelling study by Reichert and Grad, 2023 (50) addressed this question. Utilising a mathematical model, the authors examined the effects of doxycycline post-exposure prophylaxis (Doxy-PEP) on resistance dynamics and infection burden in men who have sex with men (MSM).

The model, which was a deterministic compartmental model, characterized gonorrhea transmission in a U.S. MSM population ($N = 10^6$) stratified into 3 sexual activity groups characterized by annual rates of partner change (θ). Baseline tetracycline resistance used in the model was 26.8% based on data for the prevalence of tetracycline resistance in the US MSM population derived from the 2018 Gonococcal Isolate Surveillance Project (GISP). In the model, individuals who are infected may recover naturally or by treatment with ceftriaxone monotherapy. A resistance profile was used to stratify the infections based on whether they were resistant to ceftriaxone, doxycycline, neither, or both, as well as whether they were symptomatic versus asymptomatic. The model was changed into a susceptible-exposed-infectious-susceptible (SEIS) model by adding an exposure compartment to investigate the dynamics of giving Doxy-PEP to a proportion of exposed people. Doxy-PEP was introduced at different uptake levels, ranging from 10 to 75%, after which they compared 20-year prevalence and resistance dynamics to those at baseline (i.e., no Doxy-PEP introduction).

There was an initial drop in the prevalence and incidence of gonorrhea infection with Doxy-PEP uptake.

- Within the first 5 years after Doxy-PEP introduction (at t=0), infection prevalence was reduced by up to 62%. The extent of prevalence reduction increased with higher percentage of Doxy-PEP uptake.
- The percent reduction in the cumulative number of infections after 5 years ranged from 19.5% (with10% Doxy-PEP uptake) to 49.7% (with 75% uptake) – all these relative to the existing situation of ceftriaxone monotherapy for gonorrhea treatment.
- There was also a reduction in doxycycline resistance.

As prevalence and incidence rates continued to decline with more Doxy-PEP uptake, doxycycline resistance began to increase, so that within 20 years, there was a total loss of the clinical efficacy of Doxy-PEP.

- After 20 years, the percent reduction in cumulative infections ranged from 13.5% with 10% uptake to 14.6% with 75% uptake compared with no Doxy-PEP use.
- The prevalence of doxycycline resistance also rose consistently to a threshold of 87% across all Doxy-PEP utilization levels assessed i.e. 10 – 75%.
- The time to widespread doxycycline resistance and time until strains developed double resistance to doxycycline and ceftriaxone also decreased.

This pattern was observed in all scenarios explored, such as when DOXY-PEP was only given to the highest sexual activity group which comprised 10% of the population with the remainder 90% of the population having 0% uptake.

See table 6 for details of this study.

b) What changes in tetracycline/doxycycline resistance for non-target organisms (see list above) have been modelled in the context of Doxy-PEP/PrEP use or the use of tetracyclines for the prevention of other infections?

We did not find any study addressing this research question.

c) What impacts have been modelled on the lifespan of antibiotics for target and non-target organisms (e.g. ceftriaxone for treatment of *N. gonorrhoeae*) as a result of the use of Doxy-PEP/PrEP?

The Reichert et. al. 2023, modelling study also addressed this question. They observed that Ceftriaxone monotherapy's clinically useful lifespan was not significantly extended by the use of Doxy-PEP.
The time it took for ceftriaxone resistance to reach 5% prevalence was unaffected by the introduction of DoxyPEP into the population or the degree of its use thereafter. Compared to baseline, the number of ceftriaxone treatments given decreased by more than 50% over the first five years of high DoxyPEP use (50–75%), but this reduction narrowed to 17.6% after 20 years (50). The model used a baseline tetracycline resistance of 26.8% as mentioned earlier.

d) What prescribing strategies have been modelled that maximize STI prevention outcomes and minimize AMR impact?

We did not find any study addressing this research question.

e) What changes have been modelled in emergence of antimicrobial cross-resistance (including multi-drug resistance) in the context of Doxy-PEP/Doxy-PrEP use or other prolonged or repeated doxycycline exposures, including the use of tetracyclines for the prevention of other infections (e.g. acne, malaria) on target organism (*N. gonorrhoeae*, *C. trachomatis*, and *T. pallidum*) resistance to other antimicrobials and on non-target organisms.

The above modelling study also addressed this question. They found that in the context of ceftriaxone monotherapy, and based on a baseline tetracycline resistance of 26.8%, more Doxy-PEP uptake led to continued decline in the prevalence and incidence rates of gonorrhea; but so also did doxycycline resistance begin to spread faster. So that within 20 years, there was a total loss of the clinical efficacy of Dox-PEP. The time to widespread doxycycline resistance and time until strains developed double resistance to doxycycline and ceftriaxone also decreased (50).

See table 6 for details of this study.

CONCLUSION:

This review presents evidence based on published and unpublished studies investigating the possible AMR consequences of the use of Doxy-PEP/Doxy-PrEP for the prevention of bacterial STIs. Generally, baseline vs follow up data from many of the included studies did not find any statistically significant differences in

the prevalence and incidence of tetracycline resistant strains among tetracycline exposed individuals vs non-exposed individuals.

Most of the studies that investigated the trend among target organisms (*N. gonorrhoeae* and *C. trachomatis*) and *M. genitalium* (36,37,39–41,43,44) investigated the AMR consequences of the use of Doxy-PEP for the prevention of bacterial STIs. These studies, however, were underpowered to reliably detect clinically relevant minimal differences due to typically small number of isolates. E.g. some studies were designed to conduct genomic and metagenomic analysis to examine the potential effects of antibiotics on human microbial populations (35), others were hampered by the high prevalence of background resistance (46), and yet others were not specifically assessing doxycycline/tetracycline exposures, but exposures to several antibiotics including the tetracyclines (48,51). Despite these limitations, a meta-analyses of 3 RCTs showed that participants using doxycycline or minocycline PEP had more than twice the odds (OR: 2.30; 95% CI: 0.89 – 3.35) of having tetracycline resistant gonorrhea than those in the placebo arm (26,62). Of note, resistant strains were often numerically higher in exposed individuals vs non-exposed individuals – genotypically and phenotypically. One study suggested that MIC distribution as an outcome may be more sensitive than reporting binary outcomes of resistance versus susceptible based on breakpoints (26). A modelling study did predict a future with increased prevalence of doxycycline resistant *N. gonorrhoeae* with the implementation of Doxy-PEP in a modelled MSM population (50)

Results varied in studies that looked at the trend in non-target organisms such as skin and gastrointestinal tract flora. From no significant increases in tetracycline/doxycycline resistance between baseline vs follow up data or between exposed vs non-exposed individuals (36,45) to small or transient increases (38,46) to significant increases (42,49). Tetracycline usage may promote AMR in subgingival, gastrointestinal and upper respiratory bacteria, as suggested by a systematic review of seven small prospective investigations (25).

It maybe worth noting that the pattern of doxycycline use in studies that demonstrated transient increases in tetracycline resistance during treatment, but which returned to or close to baseline after some weeks or months of treatment, differs from the intermittent ongoing use pattern as obtains in Doxy-PEP. A rise during and in the short-term after treatment might have been most relevant for the studies demonstrating transient increases.

However, given these limitations in the literature, first and foremost the lack of power to detect clinically relevant changes in resistance over time, the certainty of evidence was low to very low where a GRADE assessment was conducted, thus not providing strong or clear evidence for AMR consequences of the use of Doxy PrEP/PEP for the prevention of bacterial STIs. It is therefore likely that new evidence will influence these findings.

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This living systematic review was designed and executed by the PhD student Mary-Theresa Usuanlele and her supervisor Dr. Dominik Mertz, and in collaboration with a network of evidence-support units supported by a secretariat housed at the McMaster Health Forum**.**

Table 2: Details of studies addressing baseline and follow-up rate of tetracycline-resistance among target organisms

Study ID or Reference	**Time of Recruitment**	**Sample**	**Organism**	**Intervention**	**Comparison**	Determination of tetracycline Resistance	Outcomes	Key Findings
Luetkemeyer et. al., 2023 (36,37)	Started May, 2022 **Follow up:** 12-month	501 adults Doxy PEP n= 339 No PEP n= 162	*Neisseria gonorrhoeae* **Location:** Genital Oropharyngeal Anal Results were not disaggregated	200 mg of doxycycline within 72 hours after condomless sex	Standard care (SOC)	Tetracycline resistance: MIC≥2.0 μg/ml by agar dilution	Prevalence Resistance at baseline and end of study	Phenotypic susceptibility results in 17% (44/256) of *N. gonorrhoeae* Isolates. Month 0 (M0) TCN-R = 28.4%. After enrollment, TCN-R = 38.5%) in the doxy-PEP arm and 12.5% in the no doxy arm.
Molina et. al., 2018 (40)	July 2015 to January 2016. Follow up:	232 MSM taking PrEP against HIV, with bacterial STI in prior 12 months Doxy-PEP n =116 No PEP n = 116	*Neisseria gonorrhoeae* *Chlamydia trachomatis* **Location:** Genital Oropharyngeal Anal Results were not disaggregated	200mg Doxycycline within 24-72 hours after sex	No PEP	MICs by E-test. Resistance MICtet >1 mg/L Intermediate resistance MICtet >0·5 mg/L and ≤1 mg/L. Molecular detection of tetracycline resistance by PCR for tetM gene and the Val57Met	Tetracycline resistance in *N. gonorrhoeae*	Resistance detected in 4 *N. gonorrhoeae* isolates, while 3 *N gonorrhoeae* isolates had intermediate resistance. Molecular detection identified the tetM gene in one resistant isolates. All resistant strains also carried the

						mutation in the rpsJ gene. Overexpression of the MtrCDE-encoded efflux pump assessed by screening for mutations in the promoter of the mtrR gene or in the MtrR protein		Val57Met mutation in the rpsJ gene and mutations associated with overexpression of the antibiotic efflux pump MtrCDE.
Stewart, et. al., 2023 (44)	February 2020, to October 2022. **Follow up:** 12 months	449 non-pregnant adult women. taking PrEP against HIV Doxy-PEP: n= 224 No doxy: n = 225 group	*Neisseria gonorrhoeae* *Chlamydia trachomatis* **Location:** Genital	200mg Doxycycline within 24-72 hours after sex	No PEP	Tetracycline resistance gene tet(M) for *N. gonorrhoeae.*	Tetracycline resistance in *N. gonorrhoeae*	**At baseline:** All (100%) *N. gonorrhoeae* isolates from 16 enrollment visits were positive for tet(M) gene. **At follow up:** All (100%) *N. gonorrhoeae* isolates from 20 visits in the doxy-PEP arm were positive for tet(M) gene. All (100%) *N. gonorrhoeae* isolates from 12 visits in the no doxy arm were positive

								for tet(M) gene.
Molina et. al., 2024 (41)	January 19, 2021 to July 15, 2022 **Follow up**: Median of 9 months (IQR: 6 to 12 months)	546 (randomized); 502 (analyzed) MSM taking PrEP against HIV, with bacterial STI in prior 12 months. Doxy-PEP: n = 302 No PEP: n = 170	*Neisseria gonorrhoeae* *Chlamydia trachomatis* **Location:** Genital Throat Urine	200mg Doxycycline within 24-72 hours after sex	No PEP	MICs by E-test. Resistance MIC >0.5 mg/L High level resistance: MIC > 8 mg/L	Tetracycline resistance in *N. gonorrhoeae* and *C. trachomatis*	**At baseline:** All *N. gonorhoeae* isolates were resistant. **At follow up:** All isolates in the Doxy-PEP arm in the no PEP arm had resistance. 11 of 31 (36%) isolates in the Doxy-PEP arm and 5 of 40 in the no PEP arm had high level resistance (p = 0.043) No tetracycline resistance detected in *C. trachomatis* isolates
Vanbaelen et. al., 2024 (26)	NA **Follow up:** 1948 - 2023	3 articles met inclusion criteria	Neisseria gonorrhoeae **Location:** Not disaggregated	Tetracyclines at any dosage	Placebo or no treatment	Tetracycline resistance thresholds of (≥ 1 or 2 mg/L) as used in the in included studies in order to allow for comparison	Antimicrobial resistance (AMR) to tetracyclines in all bacterial species with available data.	No statistically significant difference in the prevalence of tetracycline resistance in *N. gonorrhoeae* between the two arms in any of the three trials

								regardless of whether resistance was defined as ≥ 1 or ≥ 2 mg/L.

Table 3: Details of studies addressing baseline rates of tetracycline/ doxycycline resistance for *M. genitalium*

Study ID or Reference	**Time of Recruitment**	**Sample**	**Organism**	**Intervention**	**Comparison**	Determination of tetracycline Resistance	Outcomes	Key Findings
Vanbaelen et. al., 2024 (26)	NA **Follow up:** 1948 - 2023	3 articles met inclusion criteria	*Neisseria gonorrhoeae* **Location:** Not disaggregated	Tetracyclines at any dosage	Placebo or no treatment	Tetracycline resistance thresholds of (≥ 1 or 2 mg/L) as used in the included studies in order to allow for comparison	Antimicrobial resistance (AMR) to tetracyclines in all bacterial species with available data.	At 6 months, there was no statistically significant difference in the prevalence of suspected tetracycline resistance between the tetracycline (1/2) and no tetracycline arms (0/3)
Bercot et. al., 2021 (39)	July 2015 to June 2016 **Follow up:** 6 months	210 asymptomatic MSM Doxy PEP n = 107 No PEP n = 103	*Mycoplasma genitalium* **Location:** Genital, anal and oropharyngeal	200 mg doxycycline within 24 hours of condomless sex (with a limit of 600 mg/week)	No prophylaxis	Detection of doxycycline-associated mutations by amplifying and sequencing the 16S rRNA gene of *Mycoplasma genitalium*	Detection of mutation of the MG 16S rRNA associated with resistance to **tetracyclines (doxycycline).**	# of isolates harbouring the 16S rRNA mutation: **At baseline:** DoxyPEP arm: 0 of 3 No Doxy PEP arm: 1 of 8 **At 6 months:** Doxy-PEP arm: 1 of 2 No Doxy PEP arm: 0 of 3.

Table 4: Details of studies addressing baseline rates of tetracycline/ doxycycline resistance among non-target organisms.

Study ID or Reference	**Time of Recruitment**	**Sample**	**Organism**	**Intervention**	**Comparison**	Determination of tetracycline Resistance	Key Findings
Jo et. al., 2021(35)	Nov. 2012 to Dec. 2018 **Follow up:** Up to 1 year	14 healthy volunteers With a minimum of 3 participants per treatment regimen.	Staphylococci **Location**: Skin	**4 standard oral regimens** **Doxycycline 20 mg twice daily for 56 days** **Doxycycline 100 mg twice daily for 56 days** Cephalexin 500mg 3 times daily for 14 days Trimethoprim/sulfamethoxazole 160/800mg twice daily for 14 days ***Purpose:*** Study aim (healthy individuals) **Outcome:** Effect of common doxycycline regimens on the emergence and selective expansion of doxycycline-resistance on Staphylococci	Untreated individuals	Addition of 2μg/ml of doxycycline to culture media to select for growth of resistant bacteria. MIC measurement for all isolates (MIC ≥ 2μg/ml) Analyses of doxycycline resistance conferring genes	Bacteria from Doxy using participants grew only in the presence of doxycycline, Doxy resistant *S.epidermidis* habored either a plasmid-associated tetK or tetL. Doxy-resistant S.epidermidis strains only isolated after doxycycline use. Doxycycline-resistant isolates from Doxy100 subjects had higher MIC values to doxycycline than those from Doxy20 subjects
Luetkemeyer et. al., 2023 (36,37)	Started May, 2022 **Follow up:** 12-month	501 adults Doxy PEP n= 339 No PEP n= 162	*S. aureus* **Location:** Genital Oropharyngeal	200 mg of doxycycline within 72 hours after condomless sex	Standard care (SOC)	Doxycycline resistance (doxy-R) for *S. aureus* defined as MIC ≥16 μg/ml by E-test	**At M0:** 215/483 (45%) of participants

			Anal Results were not disaggregated				positive for *S. aureus*. 25/215 (12%) had doxy-R *S. aureus*. **At M6:** 11/51 isolates were tet-R in doxycycline arm, and 3/29 in the soc arm **At M12:** *S. aureus* in 28% in the doxycycline group and 47% in the SoC group (P=0.03). 5/31 (16%) were tet-R in doxycycline arm and 2/28 (8%) in the soc arm.
Luetkemeyer et. al., 2023 (37)	Started May 2022 **Follow up:** 12-month	501 adults Doxy PEP n= 339 No PEP n= 162	MRSA Neisseria spp **Location:** Not disaggregated	200 mg of doxycycline within 72 hours after condomless sex **Outcome:** Prevalence of Resistance at baseline and end of study	Standard care (SOC)	Doxycycline resistance (doxy-R) for MRSA (S. aureus) defined as MIC ≥16 μg/ml by E-test and for commensal Neisseria (doxy-R: MIC ≥2 μg/ml by E-test)	M0 – MRSA cultured from 5.9% of participants. M12 - 1.5% in the doxy-PEP arm and 6.5% in the no doxy arm (p=0.077). M0 - Neisseria spp from 86.8% with doxy-R

							Neisseria in 61.8%. M12 - Neisseria spp from 85.2% in the doxy-PEP arm and 89.3% in the no doxy arm (p = 0.64), and doxy-R was 69.7% and 44.6%, respectively (p=0.017).
Grennan et. al., 2021 (43)	Unknown **Follow up:** Every 3 months for 1 year	52 HIV negative MSM or TGW on HIV PrEP and with history of syphilis	*S. aureus* **Location:** Nares	Immediate daily doxycycline 100mg for 48 weeks	Deferred doxycycline beginning 24 weeks.	Tetracycline/doxycycline resistance in *S. aureus* evaluated by Kirby-Bauer disk diffusion susceptibility test.	**Immediate arm**: Tetracycline resistance was observed in 1 of 3 *S. aureus* isolates at 24 weeks and 3 of 6 isolates at 48 weeks. **Deferred arm:** Tetracycline resistance was observed in 1 of 2 isolates at 48 weeks.
Teles et. al., 2021 (38)	January 2008 to March 2009 **Follow up:** 6 months	Patients with Stage III and Stage IV periodontitis 35 recruited and randomized	Oral microbiota **Location:** Mouth	Supra- and sub-gingival debridement done with minocycline microspheres.	Debridement done without minocycline microspheres	Samples cultivated with or without 4 μg/mL minocycline. Percentage of resistant strains determined by colony counting.	Mean % of microbiota resistant to 4 μg/mL of minocycline in the minocycline microspheres

		23 to the test group and 12 to the control group 3 lost to follow up				Taxonomy done by checkerboard DNA-DNA hybridization	group increased 1 month after the antibiotic administration period from 4.0% ± 7.0% to 17.1% ± 20%. Decreased to 12.1% ± 19.0% after 6 months after the initial local antibiotic administration (3 months after the maintenance minocycline microspheres application), Mean proportion of resistant species in subgingival plaque samples remained relatively stable over time: 2.5% ± 1.9%; 3.3% ± 4.2%; and 4.1% ± 4.2% for baseline, 1 month, and 6 months,

							respectively in control group. Same scenario in saliva samples.
Brill et. al., 2015	February 2012 to May 2013 **Follow up:** 13 weeks	Stable patients aged ≥45 years with chronic bronchitis and spirometrically confirmed COPD	Bacteria species associated with lower respiratory tract infection.	**Doxycycline 100 mg/day** Moxifloxacin 400 mg daily for 5 days every 4 weeks Azithromycin 250 mg 3 times a week	Placebo	MICs against each of the antibiotics by Etest.	Measurable increases in the degree of antibiotic resistance of isolates in all three antibiotic arms. Compared to placebo, doxycycline was associated with a factor increase in MIC of 3·74 (95% CI 1.46 to 9.58, p=0.01) Modelling the number of resistant isolates, isolates from patients in the doxycycline group were more likely to be resistant to doxycycline than placebo (OR 5.77 (95% CI 1.40

							to 23.74, p=0.02)).
Vanbaelen et. al., 2024 (26)	NA **Follow up:** 1948 - 2023	3 articles met inclusion criteria	Neisseria spp *S. aureus* **Location:** Not disaggregated	Tetracyclines at any dosage	Placebo or no treatment	Tetracycline resistance thresholds of (≥ 1 or 2 mg/L) as used in the in included studies in order to allow for comparison	Prevalence of tetracycline resistance in commensal Neisseria species was higher in the tetracycline than the placebo arm in the only study where this was assessed (OR 2.9, 95% CI 1.5-5.4). There was no statistically significant difference in the prevalence of tetracycline resistance in S. aureus between the tetracycline and placebo arms in the only study where this was assessed (OR 2.1; 95% CI 0.4-12.0)
Truong et. al., 2022 (25)	1940 - 2021	7 studies met the inclusion criteria	Various human flora spp	Doxycycline 100 -200mg/day = 5 studies	Placebo = 3 studies	Emergence of antimicrobial resistance genes.	Evidence of varying levels of tetracycline resistance at

			Location: Various anatomical sites	Tetracycline 1000mg/day = 1 study Oxytetracycline 1000,mg/day and Minocycline 100mg/day (in different intervention groups) = 1 study	Non-antibiotic controls = 3 studies Combination of placebo and alternative antibiotics = 1 study	Changes in MIC Changes in tetracycline class antibiotic susceptibility (e.g. from susceptible to intermediate and/or resistant)	baseline for both intervention and comparator arms. Five studies suggested that oral tetracycline use was associated with an increased burden of tetracycline-resistant isolates in the assessed normal flora.
Kantele et. al., 2022 (46)	2009 to 2010	412 travellers with travel history to LMICs).	E. coli ESBL-PE	Doxycycline used for the malaria prophylaxis, 46 (11.2%)	Doxycycline-nonuser	A breakpoint MIC of >4 for doxycycline and tetracycline resistance Interpreted by EUCAST guidelines.	No significant differences in ESBL-PE acquisition rates doxycycline users vs non-users (11/46 (23.9%) versus 79/366 (21.6%); p = 0.719).
Nakase et. al., 2022 (49)	2013 - 2018	Patients with acne	*S. epidermidis* **Location**: Skin	Different antibiotic classes including Tetracyclines (doxycycline and minocycline)	Non-usage	MIC CLSI and EUCAST breakpoints used for interpretation.	The data showed that the prevalence of minocycline-resistant S.

							epidermidis with tet(M) increased remarkably owing to the use of tetracyclines for acne treatment. The resistance levels to tetracyclines in S. epidermidis differed for acquisition of tet(M) and tet(K). tet(M) was significantly higher in patients who had used tetracyclines (hospital, 27.5%; clinics, 42.4%) than in patients who did not use antimicrobials ($p < 0.05$)
Mende, et. al., 2016 (45)	June 2009 and January 2012	Military personnel injured and repatriated from either Iraq or Afghanistan	S. aureus Location: groin and anterior nares	Doxycycline exposure - defined as receipt of the antibiotic after medical evacuation and prior to isolate collection	Non exposed	PCR screening for tetracycline resistance genes [tet(K), tet(L), tet(M), and tet(O)] Antimicrobial susceptibility interpreted	Of the 38 (23%) isolates resistant to the tetracycline antimicrobial class, 100%

						according to the CLSI Institute criteria	were resistant to tetracycline, 66% to doxycycline, and 61% to minocycline. tet genes, tet(M) and tet(K) contributed the highest proportion among the tetracycline resistant isolates. 17/25 isolates resistant to doxycycline, 17 (68%) were from patients with antimalarial prophylaxis.
Alkhawaja et al., 2020 (51)	6 months	Acne patients in Jordan	*C. acnes* *S. aureus* *S. epidermidis*	Antibiotics Topical - Clindamycin - Erythromycin Systemic - Doxycycline	No antibiotic	Disc diffusion assay used for Antibiotic susceptibility testing. Zones of inhibition measured in **millimeters.** Results were interpreted as resistant or sensitive according to the zone diameter interpretive chart (as per NCCLS January 2015)	There was no significant difference in the antibiotic resistance profile between the two groups (antibiotic therapy vs no antibiotic therapy); however, a higher number of

							resistant isolates was observed in the previously treated group.
Sermswan et. al., 2023, Thailand	March 2019 to April 2020	Participants aged 16 years or older with mild to severe facial Acne vulgaris (AV)	*C. acne* **Location** Skin	Antibiotic usage (including tetracycline and doxycycline) MIC breakpoints according to Clinical and Laboratory Standards Institute guidelines and European Committee on Antibiotic Susceptibility Testing	No antibiotic	Minimum inhibitory concentration (MIC) using the agar dilution method	There was no statistical difference between the prevalence of antibiotic resistance, including MDR strains, and a history of oral antibiotics (p= 0.823), topical antibiotics (p= 0.464), topical retinoids (p= 0.817), and topical BP use (p= 0.750)

Table 5: Details of studies addressing baseline and follow-up rate of non-target organism resistance to other antimicrobials, including *M. genitalium* resistance to other antimicrobials (as available: macrolides and fluoroquinolones) and rates of AMR STEI e.g. AMR *Shigella spp.*

Study ID or Reference	**Time of Recruitment**	**Sample**	**Organism**	**Intervention**	**Comparison**	Determination of tetracycline Resistance	Key Findings
Kantele et. al., 2022 (46)	2009 to 2010	412 travellers with travel	E. coli ESBL-PE	Doxycycline used for the malaria prophylaxis, 46 (11.2%)	Doxycycline-nonuser	A breakpoint MIC of >4 for doxycycline	84.4% of all ESBL-PE co-resistant to doxycycline for doxycycline users 100%

		history to LMICs).				and tetracycline resistance Interpreted by EUCAST guidelines.	(11/11) vs non-users 82.3% (65/79). No sig. diff in rates of co-resistance to ciprofloxacin, tobramycin, nitrofurantoin, and co-trimoxazole in doxycycline users vs non-users.
Mende et. al., 2016 (45)	June 2009 to January 2012	168 wounded US army personnelles repatriated through LRMC	*S. aureus*	Doxycycline exposure - defined as rseeceipt of the antibiotic after medical evacuation and prior to isolate collection	No doxycycline exposure	PMIC/ID-106 and PMIC/ID-107 panels, and interpreted according CLSI guidelines	No statistically significant difference in the overall profile of resistance to other antimicrobials between the two groups. Significantly higher proportion of isolates from doxy exposed patients vs those from non-exposed patients were resistant to levofloxacin (25% versus 10%; P = 0.016), and moxifloxacin (25% versus 10%; P = 0.016)
Truong et. al., 2022 (25)	1940 - 2021	7 studies met the inclusion criteria	Various human flora spp **Location:** Various anatomical sites	Doxycycline 100 -200mg/day = 5 studies Tetracycline 1000mg/day = 1 study Oxytetracycline 1000,mg/day and Minocycline 100mg/day (in different intervention groups) = 1 study	Placebo = 3 studies Non-antibiotic controls = 3 studies Combination of placebo and alternative antibiotics = 1 study	Emergence of antimicrobial resistance genes. Changes in MIC Changes in tetracycline class antibiotic susceptibility	All articles found evidence of varying levels of tetracycline resistance at baseline for both the intervention and comparator arm 5 studies found increased burden of tetracycline resistance in normal flora in association with the use of oral tetracyclo 3 studies found a relatively small increase in the percentage of subgingival

							flora resistant to tetracyclines during 2 weeks of antibiotic therapy 1 study found an increase in subgingival sites with tetracycline resistant Streptococcus sanguis isolates which was no longer observed at 90 days. 1 study found a decrease in the percentage of sites with tetracycline-resistant Porphyromonas gingivalis isolates, none in the percentage of sites harbouring tetracycline-resistant Aggregatibacter actinomycetemcomitans or Tannerella forsythia isolates at 12 months. 2 studies demonstrated an increase in tetracycline resistant commensal Escherichia coli in the gastrointestinal tract, 1 study reported that the number of tetracycline resistant commensal and pathogenic E. coli isolates returned to baseline 2 weeks of taking doxycycline daily for 3 weeks. 1 study saw no increase in tetracycline-resistant skin flora in the groups that took tetracyclines daily for

							18 weeks, compared with the placebo group. One study which was included in their review also met criteria for our review and the findings have been summarized above Oral tetracyclines had negligible effects on non-tetracycline resistance in Propionibacterium (Cutibacterium) species and commensal E. coli. Transient increase in multiple resistant commensal and pathogenic E. coli in stool isolates after 3 weeks of doxycycline vs no intervention. Returned to baseline 2 weeks after treatment.

Table 6: Details of studies addressing changes in tetracycline/doxycycline resistance for *N. gonorrhoeae* modelled in the context of Doxy-PEP/PrEP use or other prolonged or repeated doxycycline exposures to doxycycline, including the use of tetracyclines for the prevention or treatment of other infections.

Study ID or Reference	**Time of Recruitment**	**Sample**	**Organism**	**Intervention**	**Comparison**	Determination of tetracycline Resistance	Key Findings
Reichert and Grad, 2023 (50)	Model ran over 20-years	MSM Simulated cohort (n = 10^6) stratified	Neisseria gonorrhoeae	DoxyPEP uptake	No DoxyPEP use		Reducing the burden of gonorrhoea infection with DoxyPEP can be a useful, albeit temporary, solution.

		into 3 sexual activity groups	**Location**: Not disaggregated				Ceftriaxone's therapeutic lifespan for treating *N. gonorrhoeae* infections was not significantly affected by rising DoxyPEP uptake levels or higher initial incidence of doxycycline resistance; instead, these factors led to a quicker loss of DoxyPEP efficacy. Doxy-PEP uptake led to decline in prevalence and incidence rates of gonorrhea; but doxycycline resistance also spread faster. In 20 years, the clinical efficacy of Dox-PEP was lost. The time to widespread doxycycline resistance and time until strains developed double resistance to doxycycline and ceftriaxone also decreased.

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Appendix 1 : Detailed search strategy

Antimicrobial Resistance (AMR) Consequences of the use of Doxycycline for Prevention of Bacterial Sexually Transmitted Infections (STI): A Living Evidence Synthesis

Search Report – 02FEB2024-04JAN2024

Databases

- Medline via PubMed https://pubmed.ncbi.nlm.nih.gov/
- Embase https://www.embase.com
- Allied and Complementary Medicine Database (AMED) https://www.ebsco.com/products/research-databases/allied-and-complementary-medicine-database-amed
- Cochrane library via OVID: https://www.wolterskluwer.com/en/solutions/ovid/evidencebased-medicine-reviews-ebmr-904 (EBM Reviews – ACP Journal Club <1991 to August 2023>, Cochrane Central Register of Controlled Trials <August 2023>, Cochrane Database of Systematic Reviews <2005 to September 6, 2023>, Cochrane Clinical Answers <August 2023>, Cochrane Methodology Register <3rd Quarter 2012>, Health Technology Assessment <4th Quarter 2016>, NHS Economic Evaluation Database <1st Quarter 2016>)
- Pre-prints: https://www.preprints.org/ y https://www.medrxiv.org/

Eligible studies would include Randomized controlled trials (RCTs) including cluster RCTs, published and unpublished trials as articles, abstracts, or conference proceedings. Cohort studies, case-control and ecological studies would be included. Case reports and case series would not be eligible.

Database retrieval

Databases	HITs	DATE
PubMed	4024	02JAN2024
Embase	433	03JAN2024
Cochrane Library EBM via OVID	242	04JAN2024
ACMED via EBSCO	pending	
Pre-PRINTS – MedRxiv*	31	04JAN2024
TOTAL		

**This one is also included in Pubmed and Embase*

.ris added files -
DoxyPrEP-AMR_Pubmed_4024r_02JAN2024
DoxyPrEP-AMR_Embase_T433r_03JAN2024
DoxyPrEP-AMR_CochraneOVID_242r_04JAN2024
DoxyPrEP-AMR_Preprints_31r_04JAN2024

- **What are the possible antimicrobial resistance (AMR) consequences of the use of doxycycline for pre-exposure or post-exposure (Doxy-PrEP/Doxy-PEP) prophylaxis of bacterial sexually transmitted infections?**

	Pubmed	HITS
#1	((("Doxycycline"[MeSH Major Topic] OR "Doxycycline"[Title/Abstract] OR "Doxycycline"[Supplementary Concept] OR "tetracycline"[MeSH Major Topic] OR "tetracycline resistance"[Title/Abstract] OR "tetracycline"[Supplementary Concept]) AND ("Post-Exposure Prophylaxis"[MeSH Terms] OR "Post-Exposure Prophylaxis"[Title/Abstract] OR "postexposure prophylaxis"[Title/Abstract] OR "Pre-Exposure Prophylaxis"[MeSH Major Topic] OR "Pre-Exposure Prophylaxis"[Title/Abstract] OR "Preexposure Prophylaxis"[Title/Abstract])) **OR** (("Post-Exposure Prophylaxis"[MeSH Terms] OR "Post-Exposure Prophylaxis"[Title/Abstract] OR "postexposure prophylaxis"[Title/Abstract] OR "Pre-Exposure Prophylaxis"[MeSH Major Topic] OR "Pre-Exposure Prophylaxis"[Title/Abstract] OR "Preexposure Prophylaxis"[Title/Abstract] OR "Doxycycline"[MeSH Major Topic] OR "Doxycycline"[Title/Abstract] OR "Doxycycline"[Supplementary Concept] OR "tetracycline"[MeSH Major Topic] OR "tetracycline resistance"[Title/Abstract] OR "tetracycline"[Supplementary Concept]) AND ("bacterial resistan*"[Title/Abstract] OR "antimicrobial resistan*"[Title/Abstract] OR "antibiotic resistan*"[Title/Abstract])) **OR** (("Post-Exposure Prophylaxis"[MeSH Terms] OR "Post-Exposure Prophylaxis"[Title/Abstract] OR "postexposure prophylaxis"[Title/Abstract] OR "Pre-Exposure Prophylaxis"[MeSH Major Topic] OR "Pre-Exposure Prophylaxis"[Title/Abstract] OR "Preexposure Prophylaxis"[Title/Abstract] OR "Doxycycline"[MeSH Major Topic] OR "Doxycycline"[Title/Abstract] OR "Doxycycline"[Supplementary Concept] OR "tetracycline"[MeSH Major Topic] OR "tetracycline resistance"[Title/Abstract] OR "tetracycline"[Supplementary Concept]) AND "Sexually Transmitted"[Title/Abstract]) **OR** (("Doxycycline"[MeSH Major Topic] OR "Doxycycline"[Title/Abstract] OR "Doxycycline"[Supplementary Concept]) AND ("sexually transmitted diseases"[MeSH Terms] OR "Sexually Transmitted"[Title/Abstract] OR "STDs"[Title/Abstract])) **OR** (("drug resistance, bacterial"[MeSH Major Topic] OR "drug resistance, microbial"[MeSH Major Topic] OR "bacterial resistan*"[Title/Abstract] OR "antimicrobial resistan*"[Title/Abstract] OR "antibiotic resistan*"[Title/Abstract]) AND ("Doxycycline"[MeSH Major Topic] OR "Doxycycline"[Title/Abstract] OR "Doxycycline"[Supplementary Concept])) **OR** ((("Doxycycline"[MeSH Major Topic] OR "Doxycycline"[Title/Abstract] OR "Doxycycline"[Supplementary Concept] OR "tetracycline"[MeSH Major Topic]	**4,024**

	OR "tetracycline resistance"[Title/Abstract] OR "tetracycline"[Supplementary Concept]) AND ("bacterial resistan*"[Title/Abstract] OR "antimicrobial resistan*"[Title/Abstract] OR "antibiotic resistan*"[Title/Abstract] OR ("anti infective agents"[MeSH Major Topic] OR "anti infective agents"[Supplementary Concept])) AND ("sexually transmitted diseases"[MeSH Terms] OR "Sexually Transmitted"[Title/Abstract] OR "STDs"[Title/Abstract])) **OR** (("drug resistance, bacterial"[MeSH Major Topic] OR "drug resistance, microbial"[MeSH Major Topic] OR "bacterial resistan*"[Title/Abstract] OR "antimicrobial resistan*"[Title/Abstract] OR "antibiotic resistan*"[Title/Abstract] OR "long-term antibiotic"[Title/Abstract]) AND ("Doxycycline"[MeSH Major Topic] OR "Doxycycline"[Title/Abstract] OR "Doxycycline"[Supplementary Concept])))) AND *(y_10[Filter])*	
	EMBASE	
	('doxycycline'/exp OR doxycycline OR 'tetracycline'/exp OR 'tetracycline') AND ('post exposure prophylaxis'/exp OR 'post exposure prophylaxis' OR 'pre-exposure prophylaxis'/exp OR 'pre-exposure prophylaxis') **OR** ('doxycycline'/exp OR doxycycline OR 'tetracycline'/exp OR 'tetracycline') AND 'antibiotic resistance'/exp OR 'antibiotic resistance') AND ('sexually transmitted disease'/exp OR 'sexually transmitted disease') **OR** ('post exposure prophylaxis'/exp OR 'post exposure prophylaxis' OR 'pre-exposure prophylaxis'/exp OR 'pre-exposure prophylaxis') AND ('antibiotic resistance'/exp OR 'antibiotic resistance') AND ('sexually transmitted disease'/exp OR 'sexually transmitted disease') **AND** *(2013:py OR 2014:py OR 2015:py OR 2016:py OR 2017:py OR 2018:py OR 2019:py OR 2020:py OR 2021:py OR 2022:py OR 2023:py OR 2024:py)* ***AND*** *('article'/it OR 'article in press'/it OR 'preprint'/it OR 'review'/it)* **AND** *[embase]/lim NOT ([embase]/lim AND [medline]/lim)*	**520**
	Cochrane Library via OVID	
	(doxycycline.xm. or doxycycline.ti. or doxycycline.ab. or doxycycline.hw.) OR tetracyclines.xm. or tetracyclines.ti. or tetracyclines.ab. or tetracyclines.hw.) AND Post-Exposure Prophylaxis.xm. or Post-Exposure Prophylaxis.ti. or Post-Exposure Prophylaxis.ab. or Post-Exposure Prophylaxis.hw.) OR Pre-Exposure Prophylaxis.xm. or Pre-Exposure Prophylaxis.ti. or Pre-Exposure Prophylaxis.ab. or Pre-Exposure Prophylaxis.hw.) **OR** (doxycycline.xm. or doxycycline.ti. or doxycycline.ab. or doxycycline.hw.) OR tetracyclines.xm. or tetracyclines.ti. or tetracyclines.ab. or tetracyclines.hw.) OR Post-Exposure Prophylaxis.xm. or Post-Exposure Prophylaxis.ti. or Post-Exposure Prophylaxis.ab. or Post-Exposure Prophylaxis.hw.) OR Pre-Exposure Prophylaxis.xm. or Pre-Exposure Prophylaxis.ti. or Pre-Exposure Prophylaxis.ab. or Pre-Exposure Prophylaxis.hw.) AND bacterial resistance.xm. or bacterial resistance.ti. or bacterial resistance.ab. or bacterial resistance.hw.) OR antimicrobial resistance.xm. or antimicrobial resistance.ti. or antimicrobial resistance.ab. or antimicrobial resistance.hw.) **OR** (doxycycline.xm. or doxycycline.ti. or doxycycline.ab. or doxycycline.hw.) OR tetracyclines.xm. or tetracyclines.ti. or tetracyclines.ab. or tetracyclines.hw.) AND Post-Exposure Prophylaxis.xm. or Post-Exposure Prophylaxis.ti. or Post-Exposure Prophylaxis.ab. or Post-Exposure Prophylaxis.hw.) OR Pre-Exposure	**242**

	Prophylaxis.xm. or Pre-Exposure Prophylaxis.ti. or Pre-Exposure Prophylaxis.ab. or Pre-Exposure Prophylaxis.hw.) AND sexually transmitted diseases.xm. or sexually transmitted diseases.ti. or sexually transmitted diseases.ab. or sexually transmitted diseases.hw) **OR** (doxycycline.xm. or doxycycline.ti. or doxycycline.ab. or doxycycline.hw.) *AND* sexually transmitted diseases.xm. or sexually transmitted diseases.ti. or sexually transmitted diseases.ab. or sexually transmitted diseases.hw)	
	MedRxiv & Pre-prints	
	term ("Doxycycline" OR “tetracycline”) AND “sexually transmitted diseases” **OR** term ("Doxycycline" OR “tetracycline”) AND (“bacterial resistance” OR “antimicrobial resistance” OR “antibiotic resistance”) **OR** term ("Post-Exposure Prophylaxis" OR "Pre-Exposure Prophylaxis") AND “sexually transmitted diseases” **AND** *posted between "01 Jan, 2013 and 04 Jan, 2024"*	**31**

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	PENDIENTE	

APPENDIX 2 a

AMSTAR 2 Results for Vanbaelen et. al., 2024

Article Name: Vanbaelen et. al., 2024

Vanbaelen et. al., 2024 is a Critically Low quality review

1. Did the research questions and inclusion criteria for the review include the components of PICO?	Yes Yes Yes Yes Yes Yes
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	No
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes Yes

4. Did the review authors use a comprehensive literature search strategy?	Partial Yes Yes Yes Yes Yes
5. Did the review authors perform study selection in duplicate?	Yes Yes
6. Did the review authors perform data extraction in duplicate?	Yes Yes
7. Did the review authors provide a list of excluded studies and justify the exclusions?	No
8. Did the review authors describe the included studies in adequate detail?	Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review? **RCT**	Yes
NRSI	Yes Yes Yes Yes
10. Did the review authors report on the sources of funding for the studies included in the review?	No
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results? **RCT**	Yes
NRSI	Yes Yes Yes

12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	No
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	Yes Yes Yes
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	No
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	No
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes Yes

Appendix 2b

AMSTAR 2 Results **for Truong et. al., 2022**

Article Name: Truong et. al., 2022

Truong et. al., 2022 is a Low quality review

1. Did the research questions and inclusion criteria for the review include the components of PICO?	Yes Yes Yes Yes Yes Yes
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	YesYesYesYesYesYesYes
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Yes Yes

4. Did the review authors use a comprehensive literature search strategy?	Yes Yes Yes Yes Yes Yes Yes
5. Did the review authors perform study selection in duplicate?	Yes Yes
6. Did the review authors perform data extraction in duplicate?	Yes Yes
7. Did the review authors provide a list of excluded studies and justify the exclusions?	No
8. Did the review authors describe the included studies in adequate detail?	Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes Yes
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	
RCT	Yes
NRSI	Yes Yes Yes Yes

10. Did the review authors report on the sources of funding for the studies included in the review?	No
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	
RCT	0
NRSI	
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	0
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	Yes Yes Yes
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes Yes
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	0
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes Yes