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# A systematic review of the impacts of oral tetracycline class antibiotics on antimicrobial resistance in normal human flora

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**Objectives:** There is interest in doxycycline as prophylaxis against sexually transmitted infections (STIs), but concern about antimicrobial resistance (AMR). We conducted a systematic review (CRD42021273301) of the impact of oral tetracycline-class antibiotics on AMR in normal flora.

**Methods:** We searched MEDLINE, EMBASE, the Cochrane Library (1940–2021) and conference proceedings (2014–21) for randomized controlled trials in adults comparing daily oral tetracycline-class antibiotics to non-tetracycline controls. The primary outcome was AMR to tetracyclines; secondary outcomes included resistance to non-tetracyclines. Data were inappropriate for meta-analysis, so we analysed findings descriptively.

**Results:** Our search yielded 6265 abstracts of which 7 articles fulfilled inclusion criteria. Most were at moderate/high risk of bias, generally due to inadequate methodologic reporting. Studies used doxycycline, tetracycline, oxytetracycline or minocycline for 2–18 weeks. Most observed an increased burden of tetracycline resistance, including in subgingival ($n=3$ studies), gastrointestinal ($n=2$) and upper respiratory tract ($n=1$) flora; one study of skin flora found no change in tetracycline-resistant *Propionibacterium* species after 18 weeks of oxytetracycline/minocycline. Four studies reassessed AMR at 2–50 weeks post-intervention and reported varying degrees of resistance. Three articles reported on the prevalence of non-tetracycline AMR after doxycycline prophylaxis, of which one found a transient increase among gastrointestinal *Escherichia coli*; the other two showed no difference from control.

**Conclusions:** Although the effects are modest and transient, limited data from small prospective studies may suggest that oral tetracyclines for 2–18 weeks increase resistance in subgingival, gastrointestinal and upper respiratory tract flora. STI prophylaxis trials should include AMR in commensal bacteria as study outcomes.

## Introduction

Sexually transmitted infections (STIs), are a major global cause of morbidity and mortality.[1,2] Rates of infectious syphilis have risen in the past two decades in Canada and in other developed countries.[3] Canada's rate of new syphilis infections has dramatically increased by 167% from 2008 to 2017.[4] Increases have similarly been seen in chlamydia and gonorrhoea, with rates increasing 39% and over 96% respectively over the same interval.[4] This surge of STI transmission poses a growing threat to public health, especially among cis- and transgender women, as well as gay, bisexual and other men who have sex with men (gbMSM).[4]

The limited success of existing STI prevention strategies and the increasing presence of antimicrobial resistance (AMR) in healthcare are augmenting this global public health risk. AMR is one of the major issues facing global health in the 21st century, with many first-line antibiotics becoming less effective against common pathogens, and few new antibiotics being developed.[5] AMR presents a roadblock to treating increasingly resistant strains of organisms including *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Treponema pallidum*, as well as other community-acquired pathogens.[6,7]

Doxycycline is an oral tetracycline antibiotic that has been widely used to treat community-acquired infections and as prophylaxis against malaria and as a treatment for acne.[8–10]

The antibiotic disrupts bacterial protein synthesis by binding to the 30S ribosomal subunit in a wide range of Gram-positive and Gram-negative bacteria.[11] Other tetracycline class antibiotics such as minocycline and tetracycline have also been used to treat a wide variety of infections.[12] There is emerging interest in daily oral doxycycline for use as STI pre-exposure prophylaxis (PrEP), which is the regular use of certain medications by uninfected individuals to prevent infection before the exposure occurs, and post-exposure prophylaxis (PEP), which refers to preventative use immediately after an exposure.[13] Preliminary data suggest doxycycline PEP and PrEP could be efficacious at preventing both syphilis and chlamydia among MSM.[14–16]

To date, there have been only three small randomized studies demonstrating the potential efficacy of doxycycline STI prophylaxis, conducted among gbMSM from the US, France, and Canada, including a sub-study of the IPERGAY trial and two pilot studies.[14–16] In the earliest pilot study, HIV-positive MSM ($n=30$) either took 100 mg of doxycycline daily (PrEP) or were placed in an incentive-based financial (contingency management) control arm for remaining STI-free for 36 weeks. Participants in the PrEP arm were significantly less likely to test positive for *N. gonorrhoeae*, *Chlamydia trachomatis* or *T. pallidum* during the 48 weeks follow-up (OR = 0.27; CI: 0.09–0.83) when compared with the control arm ($P=0.02$), although the absolute number of STI outcomes was low (6 versus 15 study visits with an STI in the two arms, respectively).[14] In a sub-study of the IPERGAY trial of on-demand HIV PrEP, HIV-negative MSM ($n=232$) were randomized to receive either 200 mg of doxycycline once within 24–72 h after sex (PEP) or no intervention. This study observed a lower rate of first STI occurrence in participants taking PEP than those in the control arm (HR = 0.53; $P=0.008$, 95% CI: 0.33–0.85) over a median of 8.7 months, with 28 versus 45 observed STIs in the two arms, respectively.[15] More recently, members of our team found that daily doxycycline 100 mg was associated with a significant reduction in bacterial STIs in a pilot trial among HIV-negative gbMSM (OR = 0.18, 95% CI = 0.05–0.68); limited sampling of the nares revealed some tetracycline resistance in *Staphylococcus aureus* in both arms but data were inadequate to draw statistically meaningful conclusions.[16]

Before being widely adopted as a standard prevention strategy against STIs, the clinical impact of doxycycline prophylaxis on AMR warrants more study. There is particular concern that such use could drive tetracycline class resistance in *C. trachomatis* and *T. pallidum*, since doxycycline is the preferred treatment for urogenital, rectal and possibly pharyngeal *C. trachomatis* infections and an important treatment alternative for syphilis infection among penicillin-allergic individuals.[17–20] Similarly, prior data on long-term doxycycline as malaria prophylaxis have suggested potential associations with doxycycline resistance in nasal *S. aureus* isolates and colonization of the gastrointestinal tract with MDR coliforms, although these studies are severely limited by their observational designs.[21–24] The importance of doxycycline as a treatment option for MRSA and concerns about changes in the diversity and resistome of enteric flora mean that these issues require further study. Additional clinical trials of doxycycline-based STI prophylaxis are currently underway in Canada, the USA, Australia, Kenya and France, providing critical opportunities to address these questions with prospective data.[13] To inform these efforts, a review of the available literature may be helpful in understanding how much the use of these antibiotics as PrEP may add to the existing threat of AMR.[25–29] To address this question, we conducted a systematic review of randomized controlled trials on the impacts of oral tetracycline class antibiotics on the development of AMR in normal human flora. Because antibiotic resistance genes can be harboured on mobile genetic elements such as plasmids that may also encode resistance to other antibiotics, a secondary objective was to investigate impact on resistance to non-tetracycline class antibiotics. We also examined impact on STI incidence.

## Methods

We performed a systematic review using the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.[30] The protocol was registered on PROSPERO (CRD42021273301).

### *Eligibility criteria*

We included randomized controlled trials (1940–2021) that compared the impact of daily oral tetracycline class antibiotics versus a non-tetracycline control (placebo, no antibiotic use or alternative oral antibiotics) on the acquisition of tetracycline class AMR in normal flora among adults. We included studies that had at least one of the following outcomes for each tested bacterium and antibiotic: emergence of antimicrobial resistance genes, changes in MIC by any conventional method (e.g. Etest, Kirby–Bauer disc diffusion) or changes in reported tetracycline class antibiotic susceptibility (e.g. from susceptible to intermediate and/or resistant). We considered normal flora (specifically, bacteria that live in/on the human host without causing disease) at any anatomic site. Because antibiotic controls are expected to have different impacts on host flora AMR compared with placebo or non-antibiotic controls, we stratified results according to these two types of controls. We did not include studies that contained combination antibiotic therapy for the intervention.

### *Search strategy*

We developed an electronic database search strategy with the help of a health science librarian. Search terms were identified using synonyms, free text terms and subheadings related to tetracycline terms and clinical trial terms. We maximized sensitivity by not incorporating search terms related to our study outcomes, anticipating that many studies may have examined AMR as a secondary outcome only. The full search strategy is included in Appendix S1 (available as Supplementary data at *JAC-AMR* Online).

### *Information sources*

We searched MEDLINE, EMBASE and the Cochrane Library electronic databases on 1 February 2021. We also searched conference proceedings from 2014 to 2021 from the following meetings: Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), IDSA Annual Meeting, Infectious Disease Week, European Congress of Clinical Microbiology & Infectious Diseases (ECCMID) and the American Society of Microbiology (ASM). The reference lists of eligible studies were also used to identify articles of potential relevance. Clinical trials were also identified through the databases of ClinicalTrials.gov and the ISRCTN. There were no restrictions imposed on publication language.

### *Selection of studies*

Search results were compiled and imported into Covidence, an online program that helps in reviewing articles for systematic reviews.[31] Two independent reviewers (R.T., V.T.) assessed all identified abstracts and

**Table 1.** Criteria for assessing risk of bias

| | Assessment of risk of bias | | |
|---|---|---|---|
| Criterion | low | moderate | high |
| Randomization | • Randomization done independently and centralized<br>• Use of computer-generated sequence | • Randomization done using less rigorous system | • Randomization done using easily corruptible system |
| Concealment of treatment allocation | • Allocation of treatment concealed<br>• Performed by third party<br>• Audit trail of allocation | • Treatment allocation concealed<br>• Not performed by third party or audit trail does not exist | • Non-random allocation<br>• Method of allocation has high probability of being compromised (simple algorithm, performed by investigator, no audit trail) |
| Blinding of outcome assessment | • Analysts are blinded | • Not all analysts blinded | • No analysts blinded |
| Attrition | • No/minimal attrition (<10%)<br>• Reasons for losses to follow-up explained<br>• Account for missing data in analysis | • Moderate attrition (10%–20%)<br>• Reasons for LTFU incompletely explained | • High attrition (>20%)<br>• Reasons for LTFU not explained<br>• Ignore missing data in analysis |
| Quality of statistical analysis | • Choice of analysis type (ITT, mITT, PP) made *a priori*<br>• Analysis well explained and consistent | | • Choice of analysis type not made *a priori*<br>• Choice not well explained<br>• Analysis inconsistent with regard to type of analysis |
| Overall | • Most criteria fall under minimal risk with no more than one criterion allowed to be moderate risk or unclear | • Most criteria at minimal or moderate risk of bias with no more than one item at high risk of bias | • Not meeting criteria for minimal or moderate risk of bias |

LTFU, lost to follow-up; mITT, modified ITT.

publications that appeared to meet the inclusion criteria. Any disagreements during the review were resolved by consensus. Where consensus could not be reached, a third reviewer (D.H.S.T.) resolved the issue. For any study that lacked information required for proper assessment, we made three attempts to contact the study authors by e-mail.

### *Data extraction*

Data were extracted independently by each reviewer onto a data collection form. The primary outcome was the change 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. Secondary outcomes were changes in resistance to non-tetracycline antibiotics, using the same metrics as our primary outcome, and the incidence of bacterial STIs (syphilis, chlamydia and gonorrhoea).

### *Assessment of risk of bias*

We assessed the risk of bias among included studies using a rubric we developed from the consolidated standards of reporting trials (CONSORT) reporting checklist regarding critical methodologic features of randomized trials.[32] We prioritized an analysis of individual components of the included studies, particularly: randomization, allocation concealment, blinding of outcome assessment and attrition (Table 1). The intended purpose of these assessments was to explain any heterogeneity in results, and to perform sensitivity analyses if applicable. Quality assessors were not blinded to the articles for feasibility reasons.

### *Analysis*

The expected key measures of effect size from each study were the emergence of antimicrobial resistance genes, changes in MIC by any conventional method (e.g. Etest, Kirby–Bauer disc diffusion) or changes in tetracycline class antibiotic susceptibility. Our intention had been to meta-analyse any such quantitative findings using DerSimonian random-effects models, but the variability with which outcomes were found to be reported led us to descriptively report the findings instead.

## Results

### *Study selection*

The search strategy detected 9890 articles through MEDLINE, EMBASE and Cochrane, of which 6265 were distinct articles after deduplication (Figure 1). Through abstract screening, 122 publications appeared to meet our inclusion criteria. After full-text review, 115 articles were excluded because they did not contain our primary outcome of interest ($n=62$), did not contain our intervention of interest ($n=26$), were incomplete or did not have available results ($n=11$), did not contain our comparator groups

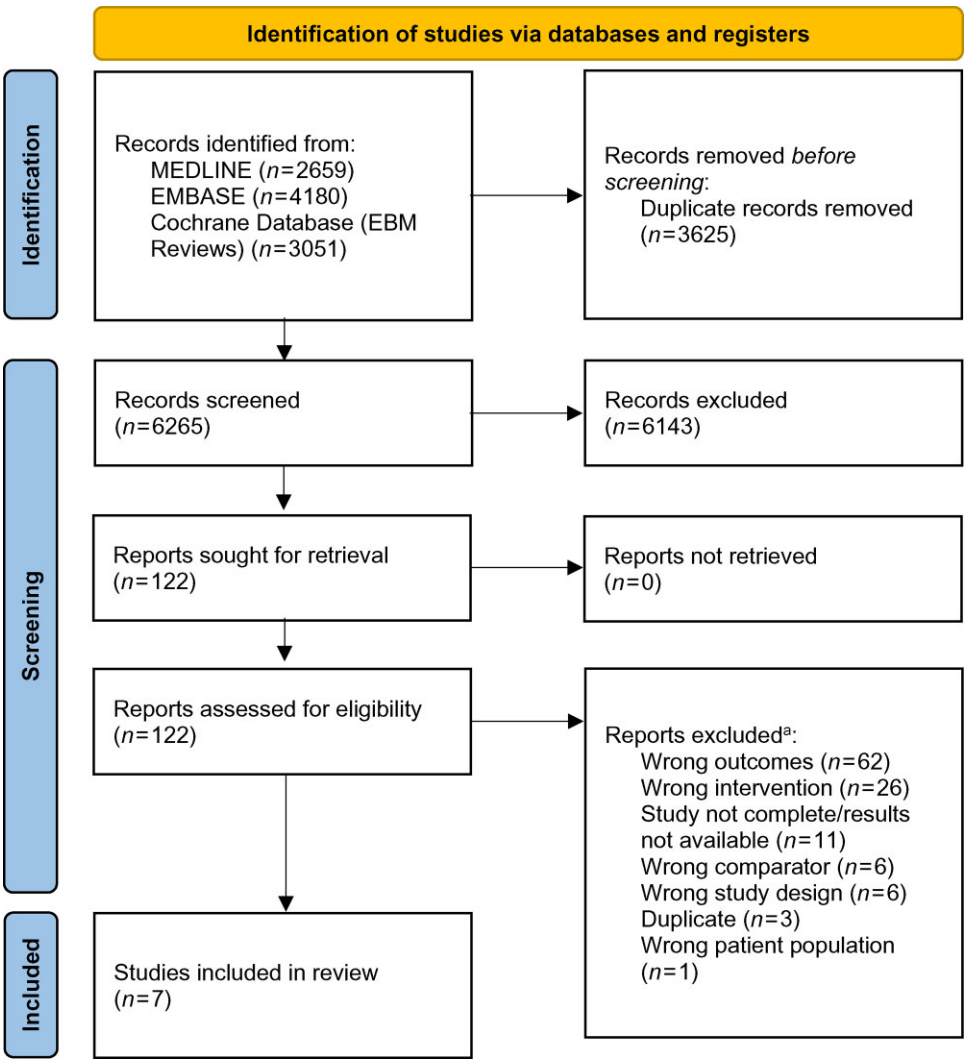


**Figure 1.** PRISMA flow chart. [a]Reasons for exclusion are mutually exclusive.

of interest ($n=6$), were not randomized controlled trials ($n=6$), were duplications of studies ($n=3$), or did not address our population of interest ($n=1$). Of the seven included articles, all reported on the burden of resistant isolates and antibiotic susceptibility, and three investigated resistance to non-tetracyclines.[23,33–38] No conference proceedings, unpublished listings in online clinical trial databases or studies in eligible reference lists were relevant. None of the seven included studies investigated the emergence of resistance genes or STI incidence.

### *Study characteristics*

The study participants came from the USA ($n=3$), UK ($n=2$), Kenya ($n=1$) or Thailand ($n=1$). The sample sizes ranged from 20 to 253. The age of participants varied greatly, from 18 to 83. The duration of treatment ranged from 2–18 weeks, with five interventions using doxycycline (total daily dose 100–200 mg/day), one using tetracycline (1000 mg/day) and one using both oxytetracycline (1000 mg/day) and minocycline (100 mg/day) as separate intervention groups. Tetracycline class antibiotics were compared with placebo ($n=3$), non-antibiotic controls ($n=3$), nothing ($n=1$) or a combination of placebo and alternative antibiotics ($n=1$). The timing of final AMR assessment varied from 3 days to 12 months since the start of treatment. The AMR outcomes were ascertained by inoculation of samples onto plates containing tetracyclines ($n=4$), by disc diffusion ($n=2$) and by Etest ($n=1$).

### *Assessment of risk of bias*

We assessed all included studies to be at either moderate or high risk of bias (Table 2), most often because inadequate reporting made the risk of bias unclear for one or more criteria. For instance, many studies did not report their method of randomization ($n=4$) or explain their concealment of treatment allocation ($n=7$). Reporting regarding the blinding of outcome assessment for included studies was also highly variable, with most articles having an unclear risk of bias for this criterion. The attrition in

**Table 2.** Assessment of risk of bias of included studies

| | Assessment of risk of bias | | | | | |
|---|---|---|---|---|---|---|
| Study | randomization | concealment of treatment allocation | blinding of outcome assessment | attrition | quality of statistical analysis | overall |
| Sack 1978[36] | High | Moderate | Low | Low | Low | Moderate |
| Arthur 1990[23] | Low | Unclear | Unclear | Low | Low | Moderate |
| Feres 1999[33] | Unclear | Unclear | Unclear | Low | Low | High |
| Feres 2002[34] | Unclear | Unclear | Unclear | Low | Low | High |
| Rodrigues 2004[35] | Unclear | Unclear | Unclear | Low | Low | High |
| Ozolins 2004[37] | Low | Moderate | Low | Moderate | Low | Moderate |
| Brill 2015[38] | Low | Unclear | High | Low | Low | Moderate |

included studies was minimal (<10%). In general, the statistical analyses were appropriately performed.

### *Studies evaluating the impact of oral tetracyclines on antibiotic resistance in human flora*

*Burden of tetracycline-resistant isolates in subgingival, gastrointestinal and skin flora*

All seven articles evaluated the impact of oral tetracyclines on outcomes relating to the burden of tetracycline-resistant isolates (Table 3).[23,33–38] All articles also suggested evidence of varying levels of tetracycline resistance at baseline for both the intervention and comparator arm.[23,33–38] In general, five studies suggested that oral tetracycline use was associated with an increased burden of tetracycline-resistant isolates in the assessed normal flora.[23,33–36] Specifically, all three studies that investigated subgingival flora ($n=20$ each) demonstrated a relatively small increase in the percentage of isolates resistant to tetracyclines during 2 weeks of antibiotic therapy.[33–35] One study saw that there was an increase in subgingival sites harbouring *Streptococcus sanguis* isolates resistant to tetracyclines, though this was no longer observed at 90 days.[33] Interestingly, Rodrigues *et al.*[35] saw a decrease in the percentage of sites harbouring tetracycline-resistant *Porphyromonas gingivalis* isolates, but no changes in the percentage of sites harbouring tetracycline-resistant *Aggregatibacter actinomycetemcomitans* or *Tannerella forsythia* isolates at 12 months.

Two studies demonstrated an increase in tetracycline-resistant commensal *Escherichia coli* in the gastrointestinal tract, based on cultures of stool specimens.[23,36] However, Sack *et al.*[36] reported that the number of both commensal and pathogenic *E. coli* isolates resistant to tetracycline returned to baseline 2 weeks after taking 100 mg (200 mg on Day 1) of doxycycline daily for 3 weeks. Surprisingly, one study in skin flora demonstrated no increase in tetracycline-resistant propionibacteria isolates in the groups that took either 1000 mg of oxytetracycline daily or 100 mg of minocycline daily for 18 weeks, compared with the placebo group.[37]

Only one article evaluated the impact of oral tetracyclines on tetracycline MICs.[38] Brill *et al.*[38] illustrated that the MIC of the upper respiratory flora significantly increased by a factor of 3.74 in individuals who took 100 mg of doxycycline daily for 13 weeks, compared with those who took placebo. It was also demonstrated that those who took this doxycycline regimen were 5.77 times more likely (95% CI: 1.40–23.74, $P=0.02$) to have doxycycline-resistant isolates than individuals who took the placebo.[38]

### *Studies evaluating the impact of oral tetracyclines on resistance to non-tetracycline antibiotics*

Three of the seven included studies evaluated the impact of oral tetracyclines on outcomes relating to non-tetracycline antibiotics (Table 4). Overall, these studies demonstrated that oral tetracyclines had negligible effects on non-tetracycline resistance in *Propionibacterium* (*Cutibacterium*) species from skin swabs and commensal *E. coli* in the gastrointestinal tract. However, one 1978 study among Peace Corps volunteers showed a transient increase in multiply resistant commensal and pathogenic *E. coli* in stool isolates after 3 weeks of doxycycline compared with no intervention, with a return to baseline 2 weeks after treatment.

## Discussion

Given emerging interest in doxycycline as STI pre- and post-exposure prophylaxis,[14] we conducted a systematic review of randomized trials evaluating the impact of oral tetracyclines on antimicrobial resistance in normal flora. We found that oral tetracyclines increased resistance to tetracycline class antibiotics in the subgingival, gastrointestinal and upper respiratory flora, but not in skin flora, when compared with non-tetracycline exposed controls. Conversely, oral tetracyclines had no significant effect on resistance to other non-tetracycline class antibiotics in commensal *E. coli* and propionibacteria (now renamed cutibacteria). There were no articles that investigated the emergence of resistance genes, or STI incidence as a secondary outcome. Due to the heterogeneity of reported outcomes, results could not be meta-analysed.

While not surprising, these observations are relevant to ongoing debates about the potential role of STI prophylaxis because tetracyclines are widely used in the treatment and prevention of a variety of common conditions, in addition to STI management. For example, doxycycline is a recommended first-line agent in the outpatient management of

**Table 3.** Impact of tetracyclines on burden of resistant isolates and antibiotic susceptibility

| | Study characteristics | | | | | | Results | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Study | population | *N* | intervention(s) | comparator(s) | follow-up | AMR method | outcome(s)[a] | intervention | comparator | difference | effect[b] |
| Studies in subgingival flora | | | | | | | | | | | |
| Feres 1999[33] and Feres 2002[34c] | Adults with periodontitis, USA | 20 | SRP + DOX 200 mg PO on Day 1, then 100 mg daily PO for the next 13 days | SRP | 3, 7, 14, 17, 21, 28 and 90 days | Inoculation onto plates containing 4 mg/L DOX | % isolates resistant to DOX in plaque | Increase at 3, 7, 14, 17 and 21 days | Minimal change over 90 days | $P < 0.05$ | ↑ |
| | | | | | | | | No increase at 28 and 90 days | Minimal change over 90 days | NOD | ↔? |
| | | | | | | | | Increase over 90 days ($P < 0.01$) | Minimal change over 90 days | OD | ↑? |
| | | | | | | | % isolates resistant to DOX in saliva | Increase at 3, 7, 14, 17, 21 and 28 days | Minimal change over 90 days | $P < 0.05$ | ↑ |
| | | | | | | | | No increase at 90 days | Minimal change over 90 days | NOD | ↔? |
| | | | | | | | | Increase over 90 days ($P < 0.01$) | Minimal change over 90 days | OD | ↑? |
| | | | | | | | % subgingival sites with DOX-resistant *S. sanguis* isolates in plaque | Increase at 14 and 17 days | Minimal change over 90 days | $P < 0.05$ | ↑ |
| | | | | | | | | No increase at 3, 7, 21, 28 and 90 days | Minimal change over 90 days | NOD | ↔? |
| Rodrigues 2004[35] | Adults with periodontitis, USA | 20 | SRP + TET 500 mg twice daily PO for 2 weeks | SRP | 1 week, 3, 6 and 12 months post-treatment | Inoculation onto plates containing 4 mg/L DOX | % isolates resistant to TET in plaque | Increase at 1 week and 6 months | Minimal change over 12 months | $P < 0.05$ | ↑ |
| | | | | | | | | No increase at 3 and 12 months | Minimal change over 12 months | NOD | ↔? |
| | | | | | | | % sites with resistant *P. gingivalis* in plaque | Decrease over 12 months ($P < 0.05$) | Minimal change over 12 months | OD | ↓? |
| | | | | | | | % sites with resistant *T. forsythia* in plaque | Minimal change over 12 months | Minimal change over 12 months | NOD | ↔? |
| | | | | | | | % sites with resistant *A. actinomycetemcomitans* in plaque | Minimal change over 12 months | Minimal change over 12 months | NOD | ↔? |
| Studies in gastrointestinal flora | | | | | | | | | | | |
| Sack 1978[36] | Peace Corps volunteers, Kenya | 39 | DOX 100 mg twice daily PO on Day 1, then daily PO for 3 weeks | Nothing | 3 and 5 weeks | Disc diffusion with 30 μg TET | % commensal *E. coli* and pathogenic *E. coli* isolates resistant to TET in stool | Increase at 3 weeks (21 → 100) | Increase at 3 weeks (6.1 → 25) | OD | ↑? |
| | | | | | | | | Decrease at 5 weeks (100 → 39) | Decrease at 5 weeks (25 → 23) | NOD | ↔? |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Arthur 1990[23] | US soldiers, Thailand | 253 | DOX 100 mg PO for 5 weeks + placebo mefloquine weekly | Oral mefloquine 250 mg weekly for 5 weeks + placebo DOX daily | 5 weeks | Disc diffusion with 30 μg TET | % individuals with TET-resistant non-ETEC isolates in stool | Increase at 5 weeks (76 → 99) | Increase at 5 weeks (69 → 86) | Difference at 5 weeks ($P$=0.01) | ↑ |
| Studies in skin flora | | | | | | | | | | | |
| Ozolins 2004[37] | Patients with acne vulgaris, UK | 391 | Oxytetracycline 500 mg twice daily PO for 18 weeks | Placebo daily PO and 5% benzoyl peroxide topical cream twice daily | 18 weeks | Inoculation onto plates containing 5 mg/L TET | Change in mean growth score for prevalence of TET-resistant propionibacteria in skin swab | No change over 18 weeks (0.0, $P$=1) | Minimal change over 18 weeks (−0.3, $P$=0.003) | No difference (NR) | ↔ |
| | | | MIN 100 mg daily PO for 18 weeks | | | | | No change over 18 weeks (0.0, $P$=1) | Minimal change over 18 weeks (−0.3, $P$=0.003) | No difference (NR) | ↔ |
| Studies in upper respiratory tract flora | | | | | | | | | | | |
| Brill 2015[38] | Patients with chronic bronchitis and COPD, UK | 49 | DOX 100 mg daily PO for 13 weeks | Placebo daily PO for 13 weeks | 13 weeks | Etest | Factor change in $MIC_{100}$ in sputum | NR | NR | 3.74, $P$=0.01, 95% CI: 1.46–9.58 | ↑ |
| | | | | | | | OR for DOX-resistant isolates in sputum | NR | NR | 5.77, $P$=0.02, 95% CI: 1.40–23.74 | ↑ |

DOX, doxycycline; ETEC, enterotoxigenic *E. coli*; MIN, minocycline; NOD, no observable difference; NR, exact values not reported; OD, observable difference; PO, oral; SRP, scaling root planning; TET, tetracycline.

[a]Unless otherwise specified, values are mean ± SD or median (IQR).

[b]Arrows indicate the direction of effect on burden of resistant isolates or antibiotic susceptibility.

[c]Studies by Feres *et al*. 2002[34] and Feres *et al*. 1999[33] have the same study sample.

**Table 4.** Change in prevalence of non-tetracycline antimicrobial resistance before and after tetracycline exposure

| | Study characteristics | | | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Study | population | *N* | intervention | comparator | follow-up | outcome | non-tetracycline antibiotics | intervention | comparator | difference | effect[a] |
| Studies in gastrointestinal flora | | | | | | | | | | | |
| Sack[36] 1978 | Peace Corps volunteers, Kenya | 39 | DOX 100 mg twice daily PO on Day 1, then daily PO for 3 weeks | Nothing | 3 and 5 weeks | % isolates of non-ETEC and ETEC in stool with resistance to multiple antibiotics | STR, sulphonamide, AMP | NR | NR | OD at 3 weeks, but NOD at 5 weeks | ↑?<br>↔? |
| Arthur[23] 1990 | US soldiers, Thailand | 253 | DOX 100 mg PO for 5 weeks + placebo mefloquine weekly | Oral mefloquine 250 mg weekly for 5 weeks + placebo DOX daily | 5 weeks | Proportion of individuals with non-ETEC strains resistant to ≥2 antibiotics in stool | AMP, CHL, ERY, GEN, KAN, neomycin, STR, SXT | Increase at 5 weeks (79% → 93%) | Increase at 5 weeks (65% → 86%) | NOD | ↔? |
| Studies in skin flora | | | | | | | | | | | |
| Ozolins 2004[37] | Patients with acne vulgaris, UK | 391 | Oxytetracycline 500 mg twice daily PO for 18 weeks | Placebo daily PO and 5% benzoyl peroxide topical cream twice daily | 18 weeks | Change in mean growth score for prevalence of resistant propionibacteria in skin swab | ERY, CLI | No increase over 18 weeks (−0.1, $P=0.362$) (−0.0, $P=1.000$) | No increase over 18 weeks (−0.5, $P<0.001$) | NOD | ↔? |
| | | | MIN 100 mg daily PO for 18 weeks | | | | | No increase over 18 weeks (−0.2, $P=0.122$) (−0.2, $P=0.085$) | No increase over 18 weeks (−0.5, $P<0.001$) | NOD | ↔? |

AMP, ampicillin; CHL, chloramphenicol; CLI, clindamycin; DOX, doxycycline; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; MIN, minocycline; NR, exact values not reported; NOD, no observable difference; OD, observable difference; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole.

[a]Arrows indicates the direction of effect on resistance.

community-acquired pneumonia, and is a useful oral agent for skin, soft tissue and some orthopaedic infections due to its activity against MRSA.[39,40] Several tetracyclines (doxycycline, minocycline, sarecycline) are used in the management of moderate-to-severe acne vulgaris, due to their activity against *Cutibacterium acnes* and relative lipophilicity, which facilitates concentration in sebaceous glands.[41] Newer agents such as tigecycline and omadacycline have broad-spectrum activity, and have been approved by the US FDA for the treatment of skin and soft tissue infections, community-acquired pneumonia and, in the case of tigecycline, complicated intra-abdominal infections as well.[42,43] Doxycycline is also effective in the prevention and treatment of Lyme disease, leptospirosis, tick-borne relapsing fever and malaria.[44–47]

Of all these clinical indications, concerns about STI prophylaxis-induced AMR may be most salient to infections of the respiratory, gastrointestinal and integumentary systems, since these conditions can arise from normal flora. It is noteworthy in this regard that for each of these particular organ systems we identified only one or two studies that reported on the AMR impacts of tetracyclines. Three other studies focused on subgingival flora in periodontitis patients. While these may be relevant to respiratory tract infections whose pathophysiology relates to micro-aspiration of oral flora, the generalizability of these studies to the general population is less certain. Given the paucity of available data, an important lesson from our review for the design of STI prophylaxis trials is thus to consider incorporating AMR monitoring among normal flora from multiple anatomic sites.

Of note was the varying burden of tetracycline resistance in normal flora present at baseline in all seven studies. Since data on prior antibiotic exposure among study participants were generally lacking, it is unclear whether this phenomenon was due to previous tetracycline use (as might be expected given that study populations included patients with periodontitis, acne vulgaris and COPD) or due to naturally occurring resistance (as has been observed among some Indigenous American groups with no prior exposure to commercial antibiotics).[48] Regardless of the cause, this observation is consistent with AMR surveillance studies showing that tetracycline resistance is not uncommon in clinical isolates. For instance, recent surveillance studies on selected European countries found that tetracycline resistance was prevalent in 66.9% and 44.9% of ESBL-producing *E. coli* and *Klebsiella* species, respectively.[49,50] In another study, global tetracycline resistance was found to be 8.7% and 24.3% for MRSA and *Streptococcus pneumoniae*, respectively.[49,51]

Potential impacts of doxycycline prophylaxis on tetracycline resistance in STI pathogens remain unclear. In the IPERGAY sub-study of doxycycline PEP, investigators assessed for AMR by performing MIC and molecular testing on *N. gonorrhoeae* isolates, as well as MIC testing on *C. trachomatis* cell cultures from PCR-positive throat and rectal samples. Although some tetracycline resistance in *N. gonorrhoeae* was observed, all fully resistant isolates were from the no-PEP group and the low yield overall limits the interpretability of the results.[15] Of note, the relatively high baseline prevalence of tetracycline resistance in that organism means that concerns have mostly focused on chlamydia and syphilis. However, the technical challenges of culturing *C. trachomatis* and *T. pallidum* are a barrier. Fortunately, resistance to tetracyclines appears to be rare in *C. trachomatis*,[52] and studies evaluating *T. pallidum* for molecular markers of doxycycline resistance have generally been negative.[53–55]

Surprisingly, although our systematic review did identify randomized trials comparing long-term doxycycline to other agents for malaria prophylaxis, none reported on AMR consequences. A potential reason is that many of these studies were conducted before the more recent era of heightened AMR awareness. Yet more recent trials evaluating long-term tetracyclines for acne treatment have also failed to study this issue, despite sometimes acknowledging AMR concerns.[56] Several clinical trials of doxycycline-based STI prophylaxis are currently underway, and it will be important that they rigorously assess for unintended consequences on AMR.[13] The inclusion of both pre- and post-exposure prophylaxis intervention arms within these trials means that data will be forthcoming on the relative impacts on resistance outcomes of consistent (in the case of daily PrEP) versus sporadic (in the case of PEP) use. However, it is important that potential future findings of adverse AMR consequences do not unintentionally limit this particular clinical indication for the same drug. This concern is particularly salient given existing discourses regarding syphilis PrEP-related stigma, including within gbMSM communities themselves.[57] AMR measures warrant inclusion as secondary outcomes in all clinical trials examining the long-term use of an antibacterial drug, regardless of the primary indication.

Strengths of our systematic review include our restriction to randomized controlled trials, our use of a deliberately broad search strategy in order to maximize sensitivity and our rigorous assessment of the risk of bias in each included study.

A few limitations also warrant mention, and may offer lessons for ongoing STI prophylaxis trials. First, we deemed all the included articles to be at moderate to high risk of bias overall, usually due to the inadequate reporting of methodologies. These inconsistencies may in part relate to the era in which they were conducted; all but one were published prior to the current CONSORT statement in 2010, and several were published even before the first iteration of CONSORT in 1996.[32,58] Second, there was significant heterogeneity in how outcomes were reported, which precluded meta-analysis. Ongoing STI prophylaxis trials have the opportunity to harmonize the definition of AMR endpoints, which could facilitate meta-analysis of resistance data in the future. Third, the interventions and AMR assessments in our systematic review only extend out to 18 and 50 weeks, respectively. The impacts of longer-term tetracycline use on AMR have not been rigorously evaluated and may represent another important opportunity for ongoing studies.

Importantly, the acceptability of doxycycline prophylaxis against STIs appears to be good. In a cross-sectional survey in gbMSM attending Toronto and Vancouver STI clinics, willingness to use was 44.1% for PrEP and 60.1% for PEP.[59] In online studies, 52.7%–75.8% of Australian gbMSM said they would be prepared to use STI PrEP, while 84% of American gbMSM expressed interest in STI PEP.[60] The numerically higher interest in PEP in these studies is of interest, as the decreased burden of drug compared with PrEP could theoretically mitigate AMR risk. Yet community members themselves have also voiced concerns about the potential for STI prophylaxis to drive AMR, emphasizing the importance of generating high-quality evidence on this question.[57]

Previous systematic reviews have unequivocally demonstrated that greater antibiotic consumption is associated with greater bacterial resistance to antibiotics.[61,62] Accordingly, we hypothesized that oral tetracyclines would directly increase tetracycline resistance in normal human flora. Our systematic review of randomized controlled trials generally confirmed this to be the case, but the limited number of included studies demonstrates a gap in our knowledge. Given the potential for normal flora to serve as a resistome reservoir for pathogenic bacteria,[63] STI prophylaxis and other trials of long-term antibiotic use should include careful evaluation of these important AMR outcomes. Such findings would need to be weighed against any potential STI prevention benefits in determining whether and how doxycycline prophylaxis should be added to the toolkit of prevention strategies. Ultimately, a modest increase in resistance among uncommon pathogens may be deemed acceptable if the STI prevention benefits of doxycycline prophylaxis are large.

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## Author contributions

All contributing authors have seen and approved the final submitted version of the manuscript. The contribution of work is as follows: D.H.S.T. and T.G. conceived the study question; D.H.S.T. designed the protocol with R.T.; R.T. and V.T. conducted database searches and screened and selected eligible articles for inclusion; R.T. and D.H.S.T. wrote the original draft of the manuscript; all authors provided input and approved the final version of the manuscript.

## Supplementary data

Appendix S1 is available as Supplementary data at *JAC-AMR* Online.

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