What is Known About Effective and Alternative Treatment Regimens for People Infected with Treponema pallidum? An Evidence Synthesis

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BACKGROUND AND RATIONALE FOR THIS EVIDENCE SYNTHESIS

Rates of infectious syphilis across Canada have been steadily rising over the last 10 years.[1] Between 2018 and 2023, rates of infectious syphilis have nearly doubled from 6,371 cases to 12,135 cases nationally (30.5 cases per 100,000 population). Syphilis continues to predominantly affect males with 64% of cases occurring in this population. However, the rate in females has been rising precipitously with national rates increasing by 204% from 2018 to 2023.[1] In turn, rates of congenital syphilis have increased across the country, with 53 cases (14.5 cases per 100,000 live births) of confirmed early congenital syphilis reported in 2023.[1] This reflects a 220% increase since 2018.

Specific populations have been disproportionately affected by the increase in infectious cases, including cisgender gay, bisexual and other men who have sex with men (GBMSM), those aged 20-39, and those experiencing health impacts and inequities related to substance use, housing instability, poverty, stigma, and systemic discrimination.[1] Additionally, in 2023, the following provinces and territories had syphilis rates higher than the national average: Northwest Territories (311.1 cases per 100,000 population), Nunavut (269.3 cases per 100,000 population), Saskatchewan (159.3 cases per 100,000 population), Yukon (132.3 cases per 100,000 population), Manitoba (103.0 cases per 100,000 population), and Alberta (51.2 cases per 100,000 population).[1]

Benzathine penicillin G 2.4 million units (MU) given intramuscularly (IM) remains the recommended treatment for syphilis. A single dose is recommended for the treatment of primary, secondary and early latent syphilis ("early syphilis"), while three weekly doses are recommended for the treatment of late latent syphilis and syphilis of unknown duration.[2] With respect to alternative treatments, doxycycline 100 mg po BID for 14 days is the recommended alternative therapy for early syphilis stages, while the course is extended to 28 days for late latent syphilis. Ceftriaxone 1 gram intravenously (IV) or IM for 10 days is only recommended in exceptional circumstances, when the standard of care is unavailable or contraindicated. There are currently no recommended alternatives to benzathine penicillin in pregnancy.[2]

Considering the increasing rates of infectious syphilis cases, their public health implications, and the risk of potential local or national benzathine penicillin shortages, there is a need for alternative and innovative approaches to the treatment of syphilis. Moreover, unforeseen public

health emergencies such as the COVID-19 pandemic may affect timely access to established parenteral first line therapies, screening, diagnosis, and treatment.

This evidence synthesis aimed to examine the existing evidence for alternative treatment regimens as compared to the current standard of care, for the treatment of primary, secondary, early latent and late latent syphilis.

Primary question: What is the effectiveness of different treatments to control early (primary, secondary, or early latent <1 year) and late (latent >1 year, or of unknown duration) syphilis, with considerations for specific population subgroups (adults, adolescents, pregnant people, lactating people, people living with HIV, people with penicillin allergy, GBMSM, Indigenous Peoples and females)?

RESEARCH METHODS:

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

Literature Search

Search Strategy:

Studies from two recently published systematic reviews (one was followed by a network meta-analysis (NMA)) were screened for eligibility.[3,4] The two reviews identified different studies, with Callado et al. 2024[3] identifying fewer references than Liu et al. 2022[4]. We updated the search from 2021 onwards to identify any relevant studies published since the completion of the search of the 2022 systematic review by Liu et al.[4]

The literature search was conducted on January 15th, 2025 using the Ovid interface for MEDLINE (Epub Ahead of Print, In-Process & Other Non-Indexed Citations, MEDLINE(R) Daily and MEDLINE(R) from 2021 to Present), and EMBASE (Appendix 1).

Selection of study/source of evidence:

After the search was completed, duplicates were removed, and all identified citations were compiled and uploaded into COVIDENCE (Victoria, Australia).

All titles/abstracts were screened by one investigator for eligibility. A second investigator reviewed all excluded hits. The same process was then completed for full-text review. Data was then extracted by one investigator and validated by a second investigator. All conflicts were resolved through discussions. Reasons for exclusion at the full text screening stage were recorded and reported.

Eligibility Criteria:

Population/Jurisdictions:

Studies including adult and adolescent participants with *Treponema pallidum* infections, stratified by those with primary vs. secondary vs. early latent (<1 year duration) vs. late latent (>1 year duration or unknown duration), where possible, were eligible. Treatment of tertiary syphilis, neurosyphilis and congenitally acquired syphilis were out of scope for this review. However, studies were included if the population meeting this exclusion criterion was <10% of the total study population, or if the data were reported in sufficient detail to exclude non-eligible patients.

Study designs:

Eligible studies include randomized controlled trials (RCTs), cohort studies, case-control studies and cross-sectional studies. Case reports and case series were not eligible. Only studies reported in English were eligible.

Interventions/Comparators:

Any study comparing two or more treatment regimens for treatment of primary, secondary, early latent or late latent syphilis, including but not limited to benzathine penicillin G, ceftriaxone, azithromycin, doxycycline, cefixime, amoxicillin, linezolid and erythromycin.

Outcomes of interest:

The primary outcome was clinical resolution of signs and symptoms of infectious syphilis combined with an adequate serologic response (“clinical cure”), or an adequate serological

response ("serological cure") of *Treponema pallidum* infection, only, as defined by the study authors. To qualify for this review, studies were required to report on the primary outcome.

Secondary outcomes:

- Transmission of *Treponema pallidum* infection to partners
- Reported antimicrobial resistance of any of the agents studied
- Participant adherence to treatment regimens
- Side-effects and reported adverse events associated with treatment, including allergies and toxicities
- HIV transmission
- Pregnancy outcomes including perinatal transmission, low birth weight, number of preterm deliveries, stillbirth/neonatal death, or clinical, laboratory, or radiologic features of congenital syphilis

Populations of special interest planned for subgroup analyses included: non-pregnant adults and adolescents, pregnant people, lactating people, people living with HIV, people with penicillin allergies, GBMSM, Indigenous Peoples, and females.

Data synthesis:

Network meta-analysis:

We conducted a network meta-analysis of the primary analysis, i.e. comparison of different regimens for the primary outcome using RCT data, only. For studies that reported on the primary outcome at different time points, we used 12 months of follow-up, as this time interval is expected to best capture clinically relevant response. If a study did not report cure at 12 months, then the time-point closest to 12 months was used. We reported summary measures as odds

ratios (OR) with corresponding 95% credible intervals (CrIs) for dichotomous outcomes and calculated absolute effects. For multi-arm trials, we combined participants randomized to different doses or durations of the same antibiotic. We used the network map command of Stata version 17.0 (StataCorp, College Station, TX) to generate network plots in which the line thickness between two nodes is proportional to the inverse variance of the effect estimate for that comparison (i.e., increased line thickness corresponds to narrower credible interval) and node diameter is proportional to the number of participants randomized to that antibiotic treatment.

The transitivity assumption gauges the appropriateness of combining evidence from individual trials with a common comparator to inform indirect evidence. The distribution of potential effect modifiers should be similar between included trials, otherwise, in the presence of large dissimilarity, network meta-analysis may be invalid.[5] The coherence assumption gauges the appropriateness of combining direct and indirect evidence and is also the result of intransitivity. We planned to assess coherence conceptually by comparing the magnitude of the effect estimates between direct and indirect evidence, along with considering the overlap in the 95% CrIs. We planned to use node splitting models to assess coherence statistically and obtain indirect effect estimates where data permitted. However, there were no comparisons to assess for incoherence (i.e., against indirect evidence).[6]

We performed Bayesian random effects network meta-analysis using non-informative priors for variance and effect parameters, and three Markov chains with 100 000 iterations after an initial burn-in of 10 000 and a thinning of 10. We assessed model convergence using the Brooks-Gelman-Rubin diagnostic test and considered convergence achieved for the comparisons where the statistic was ≤ 1.05. We conducted all NMAs using the gemtc package of R version 4.2.2

(RStudio, Boston, MA; Network meta-analysis using Bayesian methods [program]. R package version 0.8-4 version, 2020).

Traditional meta-analysis:

For observational studies, we conducted a meta-analysis when at least two included studies reported on the same comparison between two regimens and on the same primary outcome as was used in the NMA (cure rate at 12-month follow-up, or nearest time-point). We used DerSimonian and Laird random-effects meta-analyses using the metafor package in R version 4.2.2 (RStudio, Boston, MA) and summarized the effects of antibiotics on dichotomous outcomes using odd ratios (OR) and corresponding 95% confidence intervals (CI). We set the criterion for statistical significance at alpha = 0.05.

Evidence appraisal:

Risk of bias:

The risk of bias for each eligible RCT was assessed by a single reviewer and validated by a second reviewer using a revised version of the Cochrane tool for risk of bias assessment in randomized trials (RoB2).[7] Risk of bias in the following five domains was assessed: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of reported result. We rated the overall risk of bias according to the guidance in the RoB2 tool, with the following modification: for domain 5 (selection of reported result), we created a category "no information" for unregistered trials published prior to 2010, when RCT registration became mandatory. Reviewers resolved discrepancies by discussion. We also considered the risk of bias related to for-profit funding,

which we reported on narratively, but was not included in the assessment of the overall risk of bias.

For observational studies, the Newcastle Ottawa Scale was used [8]. This scale rates the risk of bias based on the key domains of selection of the study population, the comparability between groups, and the outcome. A maximum of 9 stars can be attributed to a study, reflecting the lowest risk of bias.

Grading of the evidence:

The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) framework was used to rate the certainty of evidence for each comparison analyzed in the MAs and NMA, addressing the primary outcome as high, moderate, low or very low. Ratings were validated by a second independent reviewer. Criteria to rate the certainty of evidence included considerations of risk of bias, inconsistency, indirectness, publication bias, intransitivity (NMA only), incoherence (NMA only) and imprecision, and are detailed in the Supplementary File.[9,10]

RESULTS

Search and study characteristics

Figure 1 displays the findings of the search and study inclusion process using the Preferred Reporting Items for Systematic Reviews and Meta-analyses extension for scoping review (PRISMA-ScR) flow diagram. From the previously published systematic review and the network meta-analysis, 27 possibly eligible studies were identified from Callado et al.[3] and 17 were identified from Liu et al.[4]

From the database searches from 2021 onwards, a total of 1,974 studies were identified and imported into COVIDENCE. Twenty-four duplicate studies were removed, including 16 duplicates imported from Callado et al., Liu et al.[3,4], or both, leaving 1,994 studies. These included 1,959 titles and abstracts to be screened and 35 presumptively included due to inclusion in one of the recently published review articles by Callado et al. or Liu et al.[3,4] After title and abstract screening, 47 studies were retrieved and assessed for eligibility at the full text stage, including the 35 previously identified. Three of the 35 previously identified studies were excluded as >10% of their study population had neurosyphilis and were therefore deemed out of scope for this review, and one study previously identified was excluded as it was not available in English, leaving 31 studies previously identified by Callado et al. or Liu et al.[3,4] Following full text review, 3 previously unidentified studies were added, yielding 34 studies included in this review.

Of the 34 studies that met all eligibility criteria, 14 were randomized controlled trials[11-24], 17 were retrospective cohort studies [25-41], and 3 were prospective cohort studies[42-44]. The 14 RCTs were published between 1987 and 2024 with a total of 2,280 enrolled participants (Tables 1a and

1b). The observational studies were published between 1967 and 2024 with a total of 6,895 participants (Table 2a and 2b). Most studies (30/34, 88%) used benzathine penicillin (typically 2.4 million units IM for one and up to three weekly doses) in their standard of care/control arm. In order to make meta-analysis feasible, we combined variations in duration or dosing strategies of a given antibiotic.

All studies used serology and change in titer as (part of) the primary outcome. Of note, none of the studies explicitly included the following populations of interest: pregnant individuals, lactating individuals, and Indigenous Peoples. Most studies reported the sex, and many reported the HIV status of included patients. Given the lack of data, we were unable to conduct the analysis by disease stage as planned *a priori* (i.e., primary vs secondary vs early latent vs late latent); instead we were able to conduct one post-hoc meta-analysis and NMA specifically for early syphilis (i.e., a combination of patients with primary, secondary, and early latent syphilis).

Network meta-analysis of randomized controlled trials

A total of 13/14 (93%) RCTs were eligible for the NMA, representing 1,623 participants and 10 different treatments. One RCT was ineligible as neither of the antibiotic regimens tested (amoxicillin 500mg PO tid for 14 or 28 days compared to amoxicillin 1000mg PO tid combined with probenecid 250mg PO tid for 14 or 28 days) were included in any of the other RCTs.[11]

The network generated by the NMA is shown in Figure 2. The most common antibiotics evaluated were benzathine penicillin G (772 participants; 2.4 million units IM for one and up to three weekly doses), azithromycin (401 participants; 2g PO single dose or weekly for 2 weeks), and ceftriaxone (167 participants; 1-3g IM or 1g IV single dose or up to 15 daily doses).

In comparison to the current standard of care, benzathine penicillin 2.4 million units IM once or three times weekly for early and late syphilis, respectively, the network estimates suggested higher cure rates with a combination of benzathine penicillin, followed by ceftriaxone 1g IM daily for 10 days, and then doxycycline 100mg PO BID for 20 days (OR 2.50E+15, 95%CrI 47.65-1.24E+49; Table 3). All other treatment options showed no significant difference as compared to benzathine penicillin, however, with wide credible intervals. Similarly, the combination of benzathine penicillin followed by ceftriaxone and then doxycycline is potentially superior to azithromycin 2g PO single dose or weekly for 2 weeks (OR 1.86E+15, 95%CrI 32.22-8.13E+48), procaine penicillin 2.4 MU IM once daily plus probenecid 500mg PO qid for 15 days (OR 1.23E+15, 14.46-5.09E+48), cefixime 400mg PO bid for 10 days (OR 9.66E+15, 129.13-3.89E+49), ceftriaxone 1-3g IM or 1g IV single dose or up to 15 daily doses (OR 1.63E+15, 23.14-8.14E+48), linezolid 600mg PO daily for 5 days (OR 2.65E+16, 306.32-1.25E+50), and procaine penicillin G 800,000 units daily for 15 days (OR 4.91E+15, 47.38-3.03E+49). The sample size informing these findings was very small, though, and therefore, our confidence in the findings is limited. The credible intervals crossed 1 for all other comparisons (Table 4).

Ranking of alternative regimens to the gold standard, benzathine penicillin, based on NMA results would consider the combination of benzathine penicillin followed by ceftriaxone and the doxycycline first with a probability of 98%, followed by doxycycline with a probability of 52% for second, and other penicillins combined with probenecid with a 21% probability to rank third.

Meta-analysis of observational studies

Direct meta-analysis was performed for 16 eligible observational studies. Four studies were ineligible as the antibiotic comparisons were not replicated in other studies.

As displayed in Figures 3a-h, most comparisons were between benzathine penicillin 2.4 MU IM for one to three weekly doses and various tetracyclines (see below), with no statistically significant difference: doxycycline 100mg-200mg PO bid for 14-28 days (adjusted OR 1.17, 95% CI 0.65 to 2.09; Figure 3a), doxycycline 100mg PO bid or tetracycline 500mg PO qid for 14 days (OR 1.98, 95% CI 0.82 to 4.76; Figure 3b), tetracycline 500mg PO qid for 12 days (OR 1.09, 95% CI 0.61 to 1.93; Figure 3c), and minocycline 100mg PO bid for 14 to 28 days (OR 1.20, 95% CI 0.68 to 2.10; Figure 3d). Of these, the comparison to doxycycline (Figure 3a) was the only comparison for which adjusted estimates could be used: five studies comparing benzathine penicillin 2.4 MU IM weekly for one or three doses and doxycycline 100mg PO bid for 14 or 28 days were meta-analyzed. Among these, two studies of people living with HIV adjusted for age and CD4+ count[25] and age, CD4+ count <350, prior history of syphilis, and syphilis stage.[36]

Benzathine penicillin and azithromycin 1-2g PO as single dose had similar cure rates using adjusted estimates (adjusted OR 1.00, 95% OR 0.73 to 1.37; Figure 3e): Yang et al[44] adjusted for age, stage of syphilis, initial NTT, CD4+ count, HIV viral load, combination antiretroviral therapy, and prior history of syphilis. However, there was a statistically significant difference in the cure rate in favour of benzathine penicillin in comparison to erythromycin, another macrolide antibiotic, dosed at 2g PO daily for 10-15 days (OR 6.79, 95% CI 1.55 to 29.78; Figure 3f). There was no statistically significant difference between cure rates with benzathine penicillin and

other penicillins (procaine penicillin and aqueous penicillin G) with wide confidence intervals (OR 2.19, 95% CI 0.22 to 21.73; Figure 3g), and similarly for tetracycline and erythromycin (OR 3.77, 95% CI 0.65 to 22.06; Figure 3h).

Meta-analyses by sub-group

No subgroup analysis was possible neither based on RCT nor on observational data for the following populations of special interest: non-pregnant adults and adolescents, pregnant people, lactating people, people with penicillin allergies, GBMSM, Indigenous Peoples, and females.

Subgroup meta-analysis by HIV status was not possible for RCTs, either. However, among observational studies, two studies reported outcomes for people living with HIV treated with benzathine penicillin 2.4 MU IM for one or three weekly doses versus doxycycline 100mg PO bid for 14 or 28 days, with no difference in cure rates (OR 1.03, 95% CI 0.68 to 1.57; Figure 4). Outcomes were frequently reported by primary, secondary, early latent and late latent stages of syphilis, but meta-analysis could not be performed for any one of these four categories as the available studies in each category reported unique comparisons and could not be pooled. Therefore, we opted to conduct a post-hoc analysis of early syphilis (i.e., the combination of patients with primary, secondary, and early latent syphilis) of RCT data conducting a NMA as well as meta-analysis for observational studies: A total of 12/14 (86%) RCTs reported data for patients with early syphilis, of which 11 were eligible for this post-hoc NMA. One RCT was ineligible as neither of the antibiotic regimens tested (amoxicillin 500mg PO tid for 14 or 28 days compared to amoxicillin 1000mg PO tid combined with probenecid 250mg PO tid for 14 or 28 days) were included in any of the other RCTs. The interpretation of the comparative effectiveness results for this subgroup analysis did not change when compared to the overall

NMA (see supplement file). Three RCTs reported data for patients with late syphilis.[11,13,22]. Finally, the RCT by Girma et al. included a mix of early and late syphilis patients and did not report data separately by disease stage.[14]

Among observational studies, three studies were categorized into the late syphilis category (i.e., late latent) and were not included in this post hoc subgroup analysis of early syphilis.[27,32,40] After excluding these three studies, one comparison was affected – benzathine penicillin versus azithromycin – leaving one study remaining and ineligible for meta-analysis. Therefore, the overall results from the meta-analyses above, except for the comparison benzathine penicillin versus azithromycin, reflect the comparative effectiveness of antibiotics for early syphilis as well.

Narrative description of data not included in meta-analyses

There were 5 studies that were not included in meta-analyses; 1 RCT and 4 observational studies[11,26,27,34,40].

Ando et al. performed a non-inferiority RCT that compared the treatment of adults living with HIV diagnosed with early syphilis defined as primary, secondary or early latent during the COVID-19 pandemic.[11] Patients were randomized to receive either 500mg PO tid low-dose amoxicillin monotherapy or 1000mg PO tid in combination with probenecid 250mg PO bid for 14 days for early and 28 days for late syphilis. Serologic cure at 12 months was 90.6% with the low-dose regimen and 94.4% with the combination regimen. Non-inferiority was not confirmed within 12 months (difference between groups estimate -0.04, 95% CI -0.13 to 0.05; the non-inferiority margin of -0.10 was not met). There were no significant side effects or adverse events reported.[11]

Dowell et al. conducted a retrospective cohort study comparing the treatment of 56 patients with late latent or asymptomatic neurosyphilis.[27] The proportion of neurosyphilis patients did not surpass our >10% threshold. Patients either received benzathine penicillin G 2.4 MU for three weekly doses or Ceftriaxone 1-2 grams IV daily or IM on weekdays for 10-14 days. A total of 28/43 (65%) patients in the Ceftriaxone group had an appropriate serologic response defined as a fourfold or greater decline in the serum RPR. 5 patients (12%) were serofast defined as no change in titer during the follow up period and no signs of progressive infection as defined by the authors. Nine patients (21%) had a relapse, defined as an initial fourfold decline in serum RPR, followed by a subsequent fourfold or greater rise in serum titer. One patient had treatment failure, with an increase in their titre without an initial response and was later found to have symptomatic neurosyphilis. A total of 13 patients were in the benzathine penicillin group. Similarly to above, a total of 8/13 (62%) were classified as having had an adequate serologic response, one patient was serofast by their definition, two had a relapse infection and two had treatment failure.[27]

Chen et al. conducted a retrospective cohort study in adults with early syphilis defined as primary, secondary and early latent with either a one-time dose of benzathine penicillin G 2.4 MU IM or benzathine penicillin G 2.4 MU IM along with Doxycycline 100 mg po BID over 7 days[26]. There were a total of 570 patients identified with 698 discrete syphilis episodes. Patients who received combination therapy had a significantly higher serologic response rate at 12 months in the intention-to-treat analysis compared to the monotherapy group (244/307; 79.5% compared to 275/391; 70.3%).[26] In the per-protocol analysis, the serologic response rate was 79.5% (221/278) in the combination therapy group compared to 69.9% (259/372) in those receiving benzathine penicillin alone. At the 12-month mark, there were 63 treatment failures

(20.5%) in the combination group and 116 (29.7%) in the benzathine penicillin monotherapy group. In their multivariable analysis, the independent factors associated with serologic response at 12 months were time-updated RPR titers (per 1-log2 increase, adjusted OR [aOR], 1.25; 95% CI, 1.15-1.35) and receiving combination therapy with benzathine penicillin and doxycycline (aOR, 1.71; CI 95%, 1.20-2.46). Combination therapy was associated with better outcomes as compared to benzathine penicillin monotherapy across subgroup analyses as well.[26]

Salado-Rasmussen et al. investigated serologic cures rates in patients living with HIV and early and late latent syphilis.[34] Patients either received IM penicillins: either benzathine penicillin G 2.4 MU once for early syphilis, 3 weekly doses for late syphilis, or IM procaine penicillin 600,000 IM daily for 10 days. This patient group was compared to those who received doxycycline 100 mg po BID for 14 days for early syphilis or a 30-day course of late latent syphilis. A total of 221 syphilis episodes were captured in 172 people living with HIV. Fourteen neurosyphilis cases were excluded and 202 cases were treated with either treatment modality. Overall, 126 cases were evaluated at 12 months for serologic cure. Overall, there was no significant difference between the two groups with similar rates of failure in both (12 failures in the doxycycline group (15%) and 8 cases in the penicillin group (17%); 2% difference (95% CI -1.08 - 5.08%). This corresponded to an OR of 0.78 (95% CI 0.16-3.88, p = 0.76). By logistic regression analysis, only age (OR 1.04 (95% CI 1.00-1.09) per year increment was associated with an increased risk of failure. Sex, syphilis stage, mode of acquisition or type of combination antiretroviral therapy was not associated with the outcome.[34]

Finally, Yuan et al. conducted an observational study in elderly patients defined as >60 years of age with late latent syphilis comparing benzathine penicillin G 2.4 MU once weekly for 3-4

weeks to benzathine penicillin G 2.4 MU IM once weekly for 3-4 weeks combined with Ceftriaxone 1g IM for 10 days[40]. There was no statistically significant difference in the cure rates with a numerically higher cure rate with the combination treatment in 34/38 (89%) compared to 22/32 (69%) in the monotherapy group. There was no significant difference in adverse reactions.[40]

Adverse effects

A total of 8/14 (57%) RCTs reported side effects, while only 3/20 (15%) observational studies reported adverse effects to the extent that allowed us to summarize the number of events by study group (Table 5).

The incidence of Jarish-Herxheimer reactions varied in the studies from 3.4% in the linezolid arm of the study by Ubals et al.[24] up to 33% in the ceftriaxone arm in the Cao et al. study.[12] Interestingly, the event rate in the benzathine penicillin group was also three times higher in the Cao study than in the Ubals study. The incidence of any adverse reaction (as defined by the authors) ranged from 0% in the oldest RCT conducted by Moorthy et al.[18] to 61% in the azithromycin arm in the Hook et al study.[15] In the latter study, the high event rate seems to be driven mostly by gastrointestinal symptoms. The range in event rates is most likely related to differences in definitions of adverse effects, and how thoroughly the investigators looked for and reported these adverse effects. Serious adverse effects were exceedingly rare where reported (Table 5).

EVIDENCE APPRAISAL

Risk of bias in RCTs:

The assessment of RCTs using the RoB2 for the primary outcome at 12 months are visualized in Figure 5a and 5b. The visualizations were created using the *robvis* tool.[7] All but two studies (Riedner et al. and Ubals et al.[19,24]) were found to be at 'some concern' to 'high risk' of bias. Few studies reported on the use of allocation concealment, and most studies were unblinded. Due to lack of evidence for differential intervention outside of that assigned by treatment arm, with all patients treated as outpatients, lack of blinding was not considered automatically high risk for bias. However, lack of blinding was found to be cause for 'some concern' in four studies in which different intervention arms received differential follow up, the effect of which is unclear.[12-14,23] The most common domain leading to high concern was regarding missing data, with some studies having up to 50% loss to follow-up without explanation. Outcome measurement was generally at low risk of bias, as cure was uniformly defined using objective serologic criteria. Finally, only 5 studies were registered[11,12,15,23,24]; of those, two had concern for selective outcome reporting[12,15], leading to an overall assessment of those two studies as being at high risk of bias.

Three studies disclosed donation of study drugs by pharmaceutical companies[16,18,20], and the authors of one study received research grant support from a pharmaceutical company.[16]

Certainty of Evidence (GRADE) in RCTs:

The certainty of evidence for the comparisons analyzed in the NMA ranged from low to moderate (Table 6). All but three comparisons were downgraded for serious risk of bias, and most in addition for serious imprecision. The exception to the latter were comparisons where the credible interval did not cross 1, i.e. the possibility of no effect. This was primarily the case for

comparisons that included benzathine penicillin followed by ceftriaxone and doxycycline. We also downgraded comparisons where the estimates were based on very limited data with estimates and CrIs unlikely to be plausible which occurred for several comparisons to benzathine penicillin followed by ceftriaxone and then doxycycline. We did not downgrade any of the comparisons for incoherence, inconsistency, indirectness, and publication bias.

Risk of bias in observational studies (Newcastle Ottawa Scale, NOS):

The median number of stars assigned to observational studies was 6.5 out of a maximum of 9. The studies ranged from as low as 5 to the maximum score of 9 stars in one study[41] (Table 7). While all studies received the highest number of stars for selection of the study population, lack of adjustment or matching affected the risk of bias in many studies, resulting in a lower number of stars in the comparability domain. Similarly, the scores in the outcome domain ranged from 1 to 3, mostly driven by either unexplained and significant loss of follow-up.

Certainty of Evidence (GRADE) for observational studies

The certainty of evidence was judged as very low for all the comparisons analyzed by meta-analysis (Table 8). Reasons for downgrading included risk of bias, imprecision and indirectness. We did not downgrade for inconsistency or publication bias.

KEY FINDINGS:

We identified a total of 14 RCTs and 20 observational studies to inform this evidence synthesis. A large number of different regimens have been used across studies and data on the subgroups and on secondary outcomes of interest were very limited to absent. While the definition of an adequate serological response varied, all studies included a serologic component in their definition of cure or treatment success, while some also included clinical cure criteria to the definition of the primary outcome.

Based on RCT data, one regimen appeared to be more beneficial to other regimens including the standard of care (IM benzathine penicillin typically dosed at 2.4 MU IM either once for early or three times weekly for late syphilis stages). Based on our NMA, a combination of benzathine penicillin, followed by ceftriaxone 1g IM daily for 10 days, and then doxycycline 100mg PO BID for 20 days may be superior to benzathine penicillin alone. This was informed by one single and small study (n=60 with outcome data) conducted in Italy by Drago et al. that included adult patients with syphilis across all stages (primary, secondary, early and late/unknown latent).[13] In this study, cure rates were 100% in the intervention versus only 68% in the standard of care arm. This study has several limitations including post-randomization exclusion of 9 patients in the intervention arm due to lack of eligibility, and therefore, these findings must be interpreted with caution and were rated as moderate level of evidence. Two observational studies also suggested a numerically higher cure rate with a combination of benzathine penicillin 2.4 MU IM weekly for three to four doses followed by 10 days of IM ceftriaxone[40], and benzathine penicillin 2.4 MU IM administered as a single dose followed by doxycycline 100mg PO bid for 7 days[26],

respectively. The latter study was considered at low risk of bias with 8 out of 9 stars using the Newcastle Ottawa Scale.

All other relevant comparisons did not show statistically significant differences in cure rates, and the level of evidence was mostly considered to be low due to risk of bias and serious imprecision for RCTs, and as very low for observational studies. When considering the tested regimens as potential alternatives for benzathine penicillin, the credible intervals in the NMA of RCTs and the confidence intervals in the MA of observational studies are generally too wide to conclude non-inferiority for any of these with high enough certainty of not ruling out a clinically significant difference. A notable exception is doxycycline (typically dosed at 100mg PO bid for 14 days and up to 28 days), for which the pooled point estimates were in favor in the NMA of the RCTs with an OR of 6.88 with a lower credible interval of 0.14, while the MA of observational studies using adjusted estimates where available suggested a pooled point estimate of 0.85 and a lower CI of 0.48. The estimated pooled effect sizes were in a similar ballpark for other agents within this group of antibiotics, i.e. tetracycline (typically dosed at 500mg PO qid for 12-14 days) and minocycline (typically dosed at 100mg PO bid for 14-28 days). When combining this body of evidence and the fact that doxycycline was the highest ranked option in terms of effectiveness as a benzathine penicillin sparing agent in the NMA, doxycycline remains the best supported second-line agent. However, there is still limited evidence; the comparison was graded as low, and non-inferiority cannot be safely assumed. While the final grading was higher (i.e. moderate) for the comparison to linezolid, the risk estimate was numerically clearly in favour of the standard of care with very wide CrIs, hence, despite the better grading, linezolid cannot be considered an alternative at this point of time. Another comparison to benzathine penicillin judged as being at a moderate level of evidence suggested the possibility that

azithromycin might perform as well as benzathine penicillin. Of note, in the time since those studies were performed, significant macrolide resistance has emerged in *Treponema pallidum* globally.[45] It is therefore unlikely that further studies will be performed using azithromycin for the treatment of syphilis, and its use as an alternative agent to the current gold standard cannot be recommended.

In contrast to Liu et al.[4], however, we did not find a higher cure rate with ceftriaxone (various regimens ranging from 1-3g, either administered IM or IV, and for durations ranging from single doses to up to 15 days). While our analysis did not favour benzathine penicillin over ceftriaxone either, credible intervals were very wide for this comparison, and our confidence in the findings was graded as low. It seems that the effect found in Liu et al.[4] was driven by studies in patients with neurosyphilis, a population that was out of scope of our current review.

Data on the subgroups of interest was scarce, and therefore, no conclusions could be drawn. Similarly, adverse effects have often not been reported, and when reported, definitions varied, preventing us from conducting meta-analysis. The only common theme appeared to be higher event rates of gastrointestinal symptoms with azithromycin, a finding in keeping with studies with this antibiotic for other indications.

We could not find any data in the studies included in this evidence synthesis on the following secondary outcomes of interest: transmission of *Treponema pallidum* infection to partners, reported antimicrobial resistance of any of the agents studied, participant adherence to treatment regimens, HIV transmission, and pregnancy outcomes.

In conclusion, like previously reported in Callado et al.[3], benzathine penicillin did not outperform any of the alternative regimens. While prolonged combination courses such as the use of benzathine penicillin followed by ceftriaxone and then doxycycline may be beneficial, more high-quality evidence is required, and feasibility such as adherence to these regimens must also be considered. Similarly, more research is required to safely assume non-inferiority if deviating from the current standard of care. Based on this evidence synthesis, doxycycline can be considered the best next alternative to benzathine penicillin. The absence of standardized definitions of serological cure and optimal time frames at which to assess response remains a concern and has resulted in heterogeneity across studies.

Funding Acknowledgement

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TABLES

Table 1a: Study patient characteristics - Randomized Controlled Trials

Study	Year	Country	Study population	Sample size	n female	n HIV positive	n primary syphilis	n secondary syphilis	n early latent syphilis	n late syphilis
Moorthy	1987	Singapore	Adult men with primary syphilis	20	0	0	18	0	0	0
Schofer	1989	Germany	Adults with primary or secondary syphilis	28	7	0	9	19	0	0
Rolfs	1997	USA	Adults with early syphilis	541	158	101	139	253	149	0
Hook	2002	USA	Adults with early syphilis	74	33	3	30	24	20	0
Smith	2004	USA	HIV+ asymptomatic syphilis infection	31	4	31	0	0	0	22
Riedner	2005	Tanzania	Adults with early syphilis	328	235	171	25	0	303	0
Hook	2010	USA, Madagascar	Adults with early syphilis	568	204	0	136	237	143	0

Drago	2016	Italy	Adults with syphilis	69	17	6	22	18	17	12
Cao	2017	China	Adults with early syphilis	301	123	0	45	135	50	0
Liu	2017	China	Adults with early syphilis	60	30	0	26	25	9	0
Girma	2021	Ethiopia	HIV+ adults with latent syphilis	31	9	31	0	0	0	31
Stafylis	2021	USA	HIV+ adults with early syphilis	58	0	30	2	0	28	0
Ando	2023	Japan	HIV+ adults with syphilis	112	0	112	5	57	35	15
Ubals	2024	Spain	adults with early syphilis	59	0	0	18	19	22	0

Note: differences between total sample size and the sample size by different stages can be related to a) information not provided on all patients, or not collected, b) data not provided for each subcategory, or c) small subset of patients with non-eligible disease stage (i.e. neurosyphilis)

Table 1b: Study characteristics - Randomized Controlled Trials

Study	Year	Control arm	Intervention arm	Clinical criteria for cure	Serologic criteria for cure	Max follow-up
Moorthy	1987	Benzathine penicillin G 2.4 MU IM x 1 dose	Ceftriaxone 3g IM single dose, or 2g IM daily for 2 or for 5 days	all clinical signs resolved	VDRL non-reactive within 12 months	12 months
Schofer	1989	Clemizole Penicillin G 1 million units IM daily 15d	Ceftriaxone 2g IM q2d for 4 doses	resolution of clinical symptoms + resolution of dark field positive lesions if present	VDRL ≥4 fold decrease, or VDRL non-reactive at 3 months	12 months
Rolfs	1997	Benzathine penicillin G 2.4 MU IM x 1 dose	Benzathine penicillin G 2.4 MU IM + amoxicillin 2 g po tid and probenicid 500 mg po tid x 10d	n/a	RPR ≥4 fold decrease or negative RPR with 12 months	12 months
Hook	2002	Benzathine penicillin 2.4 MU IM x1-2 weely doses	Azithromycin 2g PO single dose, or weekly over 2 weeks	resolution of clinical symptoms,	RPR ≥4 fold decrease or RPR non-reactive within 12 months	12 months
Smith	2004	Procaine penicillin IM 2.4 MU once daily +oral probenecid 500 mg po QID x 15d	Ceftriaxone 1g IM daily for 15 days	n/a	RPR ≥4 fold decrease within 12 months	12 months
Riedner	2005	Benzathine penicillin G 2.4 MU IM x 1 dose	Azithromycin 2g PO single dose	epithelialization of ulcers in 1-2 weeks, if present	RPR ≥4 fold decrease by 9 months	9 months
Hook	2010	Benzathine penicillin 2.4 MU IM x1 dose	Azithromycin 2g PO single dose		RPR ≥4 fold decrease within 6 months	6 months

Drago	2016	Benzathine penicillin 2.4 MU IM x1 dose for early, x3 weekly doses for late syphilis	Benzathine penicillin 2.4 MU IM once, then Ceftriaxone 1g IM daily x 10d, then doxycycline 100 mg BID x20d		VDRL ≥ 3-4 fold decline within 6-12 months	12 months
Cao	2017	Benzathine penicillin G IM once weekly x 2 weeks	Ceftriaxone 1g IV x10 days		RPR ≥4 fold decrease at 6-12 months	12 months
Liu	2017	Penicillin G procaine 800,000 units IM daily x15 days	Ceftriaxone 1g IV daily x10 days	resolution of skin lesions	negative non-treponemal test TRUST within 12 months	12 months
Girma	2021	Benzathine penicillin G 2.4 MU IM x3 weekly doses	Doxycycline 100mg PO for 28 days		RPR decrease ≥4 fold or non-reactive by 12-24 months	12 months
Stafylis	2021	Benzathine penicillin G 2.4 MU IM x1 dose	Cefixime 400mg PO bid x10 days		RPR decrease ≥4 fold by 3-6 months	6 months
Ando	2023	Amoxicillin 500mg PO tid x14d for early and x28d for late syphilis	Amoxicillin 1000 mg plus probenecid 250 mg PO tid x14d for early and x28 days for late syphilis		RPR ≥4 fold decrease or negative RPR within 12 months	12 months
Ubals	2024	Benzathine penicillin G 2.4 MU IM x1 dose	Linezolid 600mg PO daily x5 days	Lesion resolution by 2 weeks for primary and 6 weeks for secondary syphilis	RPR decrease ≥4 fold or non-reactive at 12, 24, 48 week assessments (3, 6, 12 months)	12 months

Table 2a: Study patient characteristics - Observational Studies

Study	Year	Study Design	Country	Study population	Sample size	n female	n HIV positive	n primary syphilis	n secondary syphilis	n early latent syphilis	n late syphilis
Lucas	1967	Prospective cohort	USA	Adults with primary or secondary syphilis	405		0				
Schroeter	1972	Prospective cohort	USA	Adults with primary or secondary syphilis	586		0				
Fiumara (1)	1977	Retrospective cohort	USA	Adolescents and adults with primary syphilis	196	22	0	196	0	0	0
Fiumara (2)	1977	Retrospective cohort	USA	Adolescents and adults with secondary syphilis	204	75	0	0	204	0	0
Fiumara (3)	1978	Retrospective cohort	USA	Adolescents and adults with early latent syphilis	275	78	0	0	0	275	0
Dowell	1992	Retrospective cohort	USA	HIV+ adults with	56	7	56	0	0	0	44

				latent syphilis							
Kiddugavu	2005	Retrospective cohort	Uganda	Adolescents and adults with syphilis	948	539	198	11	0	122	818
Ghanem	2006	Retrospective cohort	USA	Adults with early syphilis	107	60	12	21	61	25	0
Wong	2008	Retrospective cohort	Canada	Adults with primary syphilis	445	119	0	445	0	0	0
Tsai	2014	Retrospective cohort	Taiwan	HIV+ adults with early syphilis	394	0	394	35	218	141	0
Li Zheng	2014	Retrospective cohort	China	Adolescents and adults with early syphilis	748	304	0	86	334	221	
Shao	2016	Retrospective cohort	China	Adults and adolescents with early syphilis	196	103	0	52	144	0	0
Salado-Rasmussen	2016	Retrospective cohort with nested propensity-score matched case control	Denmark	HIV+ adults with syphilis	202	2	202	20	117	28	36

Yang	2016	Prospective cohort	Taiwan	HIV+ adults with early syphilis	399		399	46	166	187	0
Xiao	2017	Retrospective cohort	China	Adults with early syphilis	601	315		118	310	173	0
Antonio	2019	Retrospective cohort	Brazil	HIV+ adults with syphilis	165	1	165	4	7	91	63
Wu	2021	Retrospective cohort	China	Adults with early syphilis	276	125	0	73	95	108	0
Zengarini	2022	Retrospective cohort	Italy	Adults with syphilis infection	82	9	35	14	18	16	34
Yuan	2023	Retrospective cohort	China	Adults >60 years old with latent syphilis	70	20	0	0	0	0	70
Chen	2024	Retrospective cohort	Taiwan	HIV+ adults with early syphilis	698 (episodes)	1	570	0	168	530	0

Table 2b: Study characteristics - Observational Studies

Study	Year	Control arm	Intervention arm	Clinical criteria for cure	Serologic criteria for cure	Max follow-up
Lucas	1967	Benzathine penicillin G 2.4 MU IM x 1 dose	various agents including procaine penicillin, aqueous penicillin, tetracycline, erythromycin, spectinomycin over 8-10 days	persistent symptom resolution	VDRL response within the first year, with no relapse	12 months
Schroeter	1972	Benzathine penicillin G 2.4 MU IM x 1 dose	various agents including procaine penicillin, aqueous penicillin, tetracycline, erythromycin over 8-10 days	persistent symptom resolution	VDRL response within the first year, with no relapse	24 months
Fiumara (1)	1977	Benzathine penicillin G 2.4 MU IM x2 weekly doses	Tetracycline 500mg PO qid x12d	lesions healed	Seronegative within 12 months (RPR)	12 months
Fiumara (2)	1977	Benzathine penicillin G 2.4 MU IM x2 weekly doses	Tetracycline 500mg PO qid x12d		RPR non-reactive within 2 years	24 months
Fiumara (3)	1978	Benzathine penicillin G 2.4 MU IM x2 weekly doses	Tetracycline 500mg PO qid x12d		RPR ≥4 fold decrease in 1 year, AND non-reactive within 4y	4 years
Dowell	1992	Benzathine penicillin G 2.4 MU IM x3 weekly doses	Ceftriaxone 1-2g IV or IM daily x10-14d		RPR ≥4 fold decrease or serofast over 6+ months	6+ months
Kiddugavu	2005	Benzathine penicillin G 2.4 MU IM x1 dose	Azithromycin 1g PO single dose with or without benzathine penicillin		RPR ≥4 fold decrease or negative RPR after 10 months, or 4 fold reduction in TRUST assay	10+ months
Ghanem	2006	Benzathine penicillin G 2.4 MU IM x1 dose	Doxycycline 100mg PO bid x14d		RPR ≥4 fold decrease at 270-400 days	13 months

Wong	2008	Benzathine penicillin G 2.4 MU IM x1 dose	Doxycycline 100mg PO bid or tetracycline 500mg PO qid x14d		RPR decrease ≥4 fold by 6 months, or ≥8 fold by 12 months, or ≥16 fold by 24 months; or stable titers +/- 1 dilution if baseline RPR ≤ 1:4	12 months
Tsai	2014	Benzathine penicillin G 2.4 MU IM x1 dose	Doxycycline 100mg PO bid x14d		RPR ≥4 fold decrease at 6-12 months	12 months
Li Zheng	2014	Benzathine penicillin G 2.4 MU IM x2 weekly doses	Doxycycline 200mg PO bid or tetracycline 500mg PO qid x14d		RPR ≥4 fold decrease or negative RPR at 6 months; if initial RPR≤1:2, +/- 1 dilution is acceptable	6 months
Shao	2016	Benzathine penicillin G 2.4 MU IM x1 dose	Minocycline 100mg PO bid x14, or x28d	no clinical symptoms	nontreponemal titers non-reactive within 24 months (RPR)	24 months
Salado-Rasmussen	2016	Benzathine penicillin G 2.4 MU IM x1 for early and x3 weekly for late syphilis; or IM procaine penicillin 600,000 IM daily x10d	Doxycycline 100mg PO bid x14d for early and x30d for late syphilis		RPR ≥4 fold decrease within 12 months	12 months
Yang	2016	Benzathine penicillin G 2.4 MU IM x1 dose	Azithromycin 2g PO x1 dose		RPR decrease ≥4 fold by 12 months	12 months
Xiao	2017	Benzathine penicillin G 2.4 MU IM x1 dose	Doxycycline 100mg PO bid x14d	Resolution of lesions if present	If baseline RPR≥1:8, ≥4 fold decline by 6-12 months; If baseline RPR<1:8, RPR negative	12 months
Antonio	2019	Benzathine penicillin G 2.4 MU IM x1 for early and x3 weekly doses for late syphilis	Doxycycline 100mg PO bid x14d for early and x28d for late syphilis		VRDL non-reactive or ≥4 fold decrease at 6-12 months	12 months

Wu	2021	Benzathine penicillin G 2.4 MU IM x1-2 weekly doses	Minocycline 100mg PO bid x28d		RPR non-reactive or ≥4 fold decrease over 24 months	24 months
Zengarini	2022	Benzathine penicillin G 2.4 MU IM x1 for early and x3 weekly doses for late syphilis	Doxycycline 100mg PO bid x14d for early and x28d for late syphilis		RPR decrease ≥4 fold or non-reactive by 24 months	24 months
Yuan	2023	Benzathine penicillin G 2.4 MU IM x3-4 weekly doses	Same plus Ceftriaxone 1g IM daily x10d		"Significantly effective" - NTT decrease ≥4 fold by 6-12 months; "effective" NTT decline 2 fold; non-reactive NTT [though RPR stated in their cure definition, TRUST used]	12 months
Chen	2024	Benzathine penicillin G 2.4 MU IM x1 dose	Same plus doxycycline 100mg PO bid x7d		RPR ≥4 fold decrease within 12 months	12 months

Table 3: Network estimates with comparison to benzathine penicillin for the primary outcome at 12 month follow up (or nearest time-point).

	Point estimate	CrI lower limit	CrI upper limit
Penicillins (procaine or aqueous penicillin G)	0.65	0.01	21.25
Benzathine penicillin followed by ceftriaxone and doxycycline	2.50E+15	47.65	1.24E+49
Procaine penicillin plus probenecid	2.15	0.02	158.87
Doxycycline	6.88	0.14	625.93
Benzathine penicillin followed by amoxicillin plus probenecid	1.11	0.04	29.34
Azithromycin	1.23	0.14	14.29
Ceftriaxone	1.73	0.11	15.87
Linezolid	0.11	0	3.87
Cefixime	0.28	0.01	7.62

Table 4: Network estimates with comparison to antibiotics other than benzathine penicillin for cure rates at 12 month follow up (or nearest time-point).

Intervention	Reference	Point estimate	CrI lower limit	CrI upper limit
CRO	AZM	1.39	0.03	28.53
PEN	AZM	0.52	0	26.69
PEN_PRO	AZM	1.69	0.01	212.97
DOX	AZM	5.34	0.06	768.57
BENPEN_AMX_PRO	AZM	0.89	0.01	45.98
BENPEN_CRO_DOX	AZM	1.86E+15	32.22	8.13E+48
CEFIXIME	AZM	0.23	0	11.85
LNZ	AZM	0.09	0	5.8
BENPEN_CRO_DOX	BENPEN_AMX_PRO	2.34E+15	34.56	1.18E+49
CEFIXIME	BENPEN_AMX_PRO	0.26	0	25.78
DOX	BENPEN_AMX_PRO	6.28	0.04	1530.35
LNZ	BENPEN_AMX_PRO	0.1	0	11.97
PEN	BENPEN_AMX_PRO	0.6	0	58.55
PEN_PRO	BENPEN_AMX_PRO	1.93	0.01	391.02
CRO	BENPEN_AMX_PRO	1.58	0.02	71.31
CRO	BENPEN_CRO_DOX	0	0	0.04
DOX	BENPEN_CRO_DOX	0	0	0.23
LNZ	BENPEN_CRO_DOX	0	0	0
CEFIXIME	BENPEN_CRO_DOX	0	0	0.01
PEN_PRO	BENPEN_CRO_DOX	0	0	0.07
PEN	BENPEN_CRO_DOX	0	0	0.02
CRO	CEFIXIME	6.12	0.07	300.33
DOX	CEFIXIME	24.6	0.15	6654.94
PEN	CEFIXIME	2.31	0.01	222.12
PEN_PRO	CEFIXIME	7.53	0.02	1700.92
LNZ	CEFIXIME	0.39	0	52.09
DOX	CRO	4.07	0.05	846.97
LNZ	CRO	0.07	0	6.51
PEN_PRO	CRO	1.26	0.03	56.94
PEN	CRO	0.39	0.01	6.77
LNZ	DOX	0.02	0	3.11
PEN	DOX	0.09	0	16.34
PEN_PRO	DOX	0.29	0	95.39

PEN	LNZ	5.89	0.01	845.92
PEN_PRO	LNZ	19.64	0.05	5364.38
PEN_PRO	PEN	3.2	0.03	734.61

AZM, azithromycin. BENPEN, benzathine penicillin. AMX, amoxicillin. PRO, probenecid. CRO, ceftriaxone. DOX, doxycycline. LNZ, linezolid. PEN, penicillins other than benzathine penicillin.

Table 5: Adverse effects

Study	Year	Any side effects/drug reactions	Serious adverse effects	Jarish-Herxheimer	Allergic reactions	Notes
RCTs						
Moorthy	1987	0 across all study arms				
Schofer	1989				1 in clemizole penicillin arm	
Hook	2002			5/21 (24%) in benzathine pencillin arm vs. 9/53 (17%) in the two azithromycin arms combined		
Hook	2010	132/285 (46%) in benzathine penicillin vs. 174/283 (61%) in azithromycin arm	10/285 (0.4%) in benzathine penicillin arm vs. 8/283 (0.03%) in azithromycin arm			Difference in any side effects seems driven by GI upset (7% vs. 24%) and CNS symptoms (2.4% vs. 6.7%)
Cao	2017		0 in both arms	37/118 (31%) in the benzathine pencillin arm vs. 46/112 (33%) in ceftriaxone arm		
Girma	2021		0 in both arms			
Ando	2023	10/56 (18%) in amoxicillin vs. 14/56 (25%) in amoxicillin plus probenecid arm		7/56 (12.5%) in amoxicillin vs. 8/56 (14%) in amoxicillin plus probenecid arm		Adverse effects: 3 cases of diarrhea in amoxicillin plus probenecid arm vs. no cases in amoxicillin arm

Ubals	2024	5/30 (17%) in benzathine penicillin vs. 5/29 (17%) in linezolid arm		3/30 (10%) in benzathine penicillin vs. 1/29 (3.4%) in linezolid arm		Adverse effects mostly GI symptoms with 2 and 3 cases, respectively
Observational studies						
Lucas	1967	0 to 1 resulting in discontinuation across all study arms				
Schroeter	1972	1.6% in benzathine penicillin vs. 9% in procaine penicillin vs. 14% in erythromycin vs. 0% in other study arms				
Yuan	2023	4/32 (13%) in benzathine penicillin arm vs. 6/38 (16%) in the arm combined with ceftriaxone				Adverse effects primarily GI symptoms (2 and 3 cases, respectively)

Table 6. Certainty of Evidence of RCTs in the NMA - GRADE assessment

COMPARISON		GRADE				BEST ESTIMATE	REASON FOR DOWNGRADING
Intervention	Reference	Starting rating	Incoherence	Imprecision	Final rating		
CRO	AZM	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN	AZM	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN_PRO	AZM	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
DOX	AZM	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
BENPEN_AMX_PRO	AZM	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
BENPEN_CRO_DOX	AZM	Moderate	Not serious	Not serious	Moderate	NMA	Serious risk of bias
CEFIXIME	AZM	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
LNZ	AZM	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
LNZ	BENPEN	High	Not serious	Serious	Moderate	NMA	Serious imprecision
PEN	BENPEN	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN_PRO	BENPEN	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision

CRO	BENPEN	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
BENPEN_AMX_PRO	BENPEN	High	Not serious	Serious	Moderate	NMA	Serious imprecision
AZM	BENPEN	High	Not serious	Serious	Moderate	NMA	Serious imprecision
CEFIXIME	BENPEN	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
DOX	BENPEN	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
BENPEN_CRO_DOX	BENPEN	Moderate	Not serious	Not serious	Moderate	NMA	Serious risk of bias
BENPEN_CRO_DOX	BENPEN_AMX_PRO	Moderate	Not serious	Not serious	Moderate	NMA	Serious risk of bias
CEFIXIME	BENPEN_AMX_PRO	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
DOX	BENPEN_AMX_PRO	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
LNZ	BENPEN_AMX_PRO	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN	BENPEN_AMX_PRO	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN_PRO	BENPEN_AMX_PRO	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
CRO	BENPEN_AMX_PRO	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
CRO	BENPEN_CRO_DOX	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision

DOX	BENPEN_CRO_DOX	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
LNZ	BENPEN_CRO_DOX	Moderate	Not serious	Not serious	Moderate	NMA	Serious risk of bias
CEFIXIME	BENPEN_CRO_DOX	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN_PRO	BENPEN_CRO_DOX	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN	BENPEN_CRO_DOX	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
CRO	CEFIXIME	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
DOX	CEFIXIME	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN	CEFIXIME	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN_PRO	CEFIXIME	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
LNZ	CEFIXIME	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
DOX	CRO	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
LNZ	CRO	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN_PRO	CRO	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN	CRO	High	Not serious	Serious	Moderate	NMA	Serious imprecision

LNZ	DOX	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN	DOX	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN_PRO	DOX	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN	LNZ	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN_PRO	LNZ	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision
PEN_PRO	PEN	Moderate	Not serious	Serious	Low	NMA	Serious risk of bias, serious imprecision

AZM, azithromycin. BENPEN, benzathine penicillin. AMX, amoxicillin. PRO, probenecid. CRO, ceftriaxone. DOX, doxycycline. LNZ, linezolid. PEN, penicillins other than benzathine penicillin.

Table 7: Risk of Bias in observational studies - Newcastle Ottawa Scale

Author, year	Selection	Comparability	Outcome	Total stars
Lucas 1967	4	0	2	6
Schroeter 1972	4	0	2	6
Fiumara 1977 (primary)	4	0	3	7
Fiumara 1977 (secondary)	4	0	3	7
Fiumara 1978	4	0	3	7
Dowell 1992	4	0	1	5
Kiddugavu 2005	4	1	2	7
Ghanem 2006	4	1	1	6
Wong 2008	4	0	2	6
Tsai 2014	4	2	2	8
Li Zheng 2014	4	0	1	5
Shao 2016	4	0	2	6
Salado-Rasmussen 2016	4	2	2	8
Yang 2016	4	2	3	9
Xiao 2017	4	0	2	6
Antonio 2019	4	2	1	7
Wu 2021	4	0	2	6
Zengarini 2022	4	2	3	9
Yuan 2023	4	0	2	6
Chen 2024	4	2	2	8

Table 8. Certainty of Evidence of non-randomized studies in meta-analyses for the primary outcome - GRADE assessment

Reference	Comparator	Risk of Bias	Inconsistency	Imprecision (85% cutoff)	Indirectness	Publication Bias	GRADE rating
Benzathine penicillin	Azithromycin	Not serious	Not serious	Serious	Serious	Not serious	Very low
Benzathine penicillin	Doxycycline	Not serious	Not serious	Serious	Serious	Not serious	Very low
Benzathine penicillin	Doxycycline or tetracycline	Serious	Not serious	Serious	Not serious	Not serious	Very low
Benzathine penicillin	Erythromycin	Serious	Not serious	Not serious	Serious	Not serious	Very low
Benzathine penicillin	Minocycline	Serious	Not serious	Serious	Not serious	Not serious	Very low
Benzathine penicillin	Procaine or aqueous penicillin G	Serious	Not serious	Serious	Not serious	Not serious	Very low
Benzathine penicillin	Tetracycline	Not serious	Not serious	Serious	Not serious	Not serious	Very low
Tetracycline	Erythromycin	Serious	Not serious	Serious	Serious	Not serious	Very low

FIGURES

Figure 1. Flow diagram for the preferred reporting system for systematic reviews and meta-analysis (PRISMA).

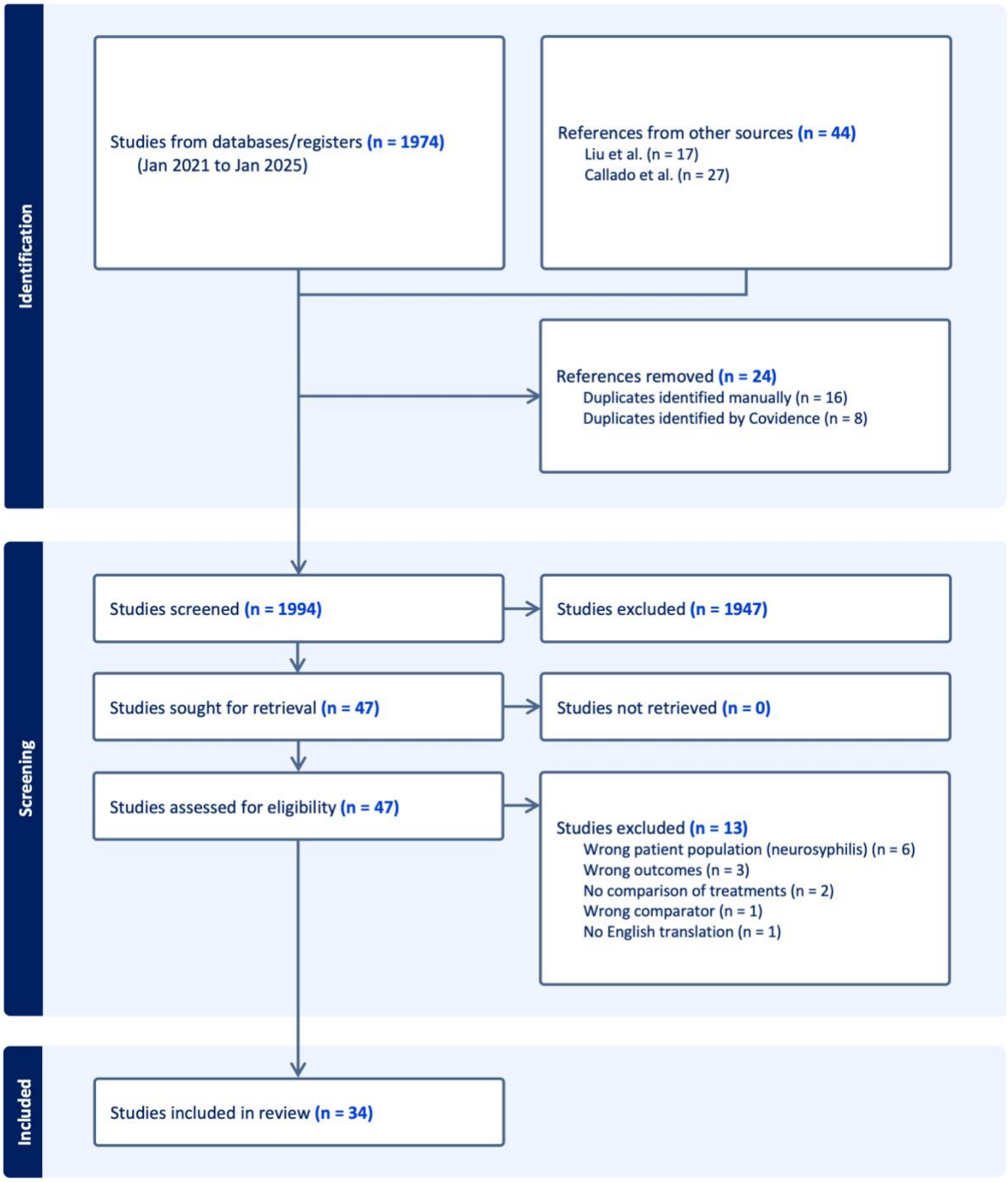
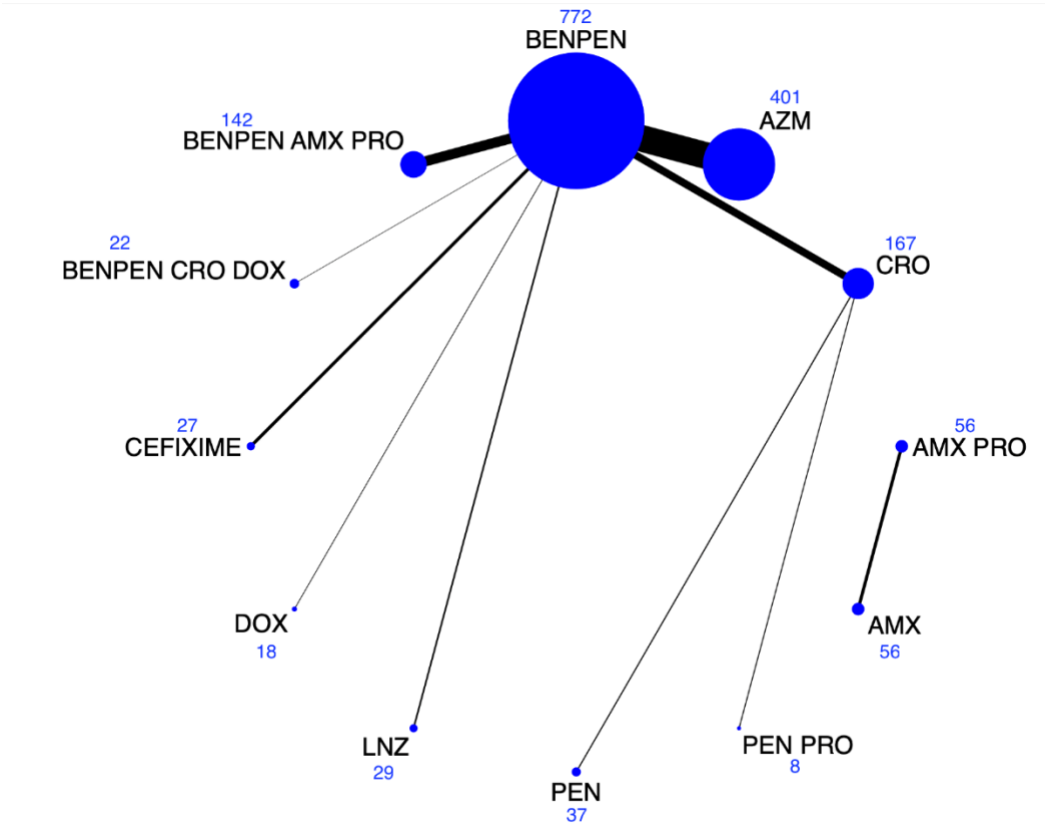


Figure 2. Network diagram of cure rates at 12 month follow up (or nearest time-point).



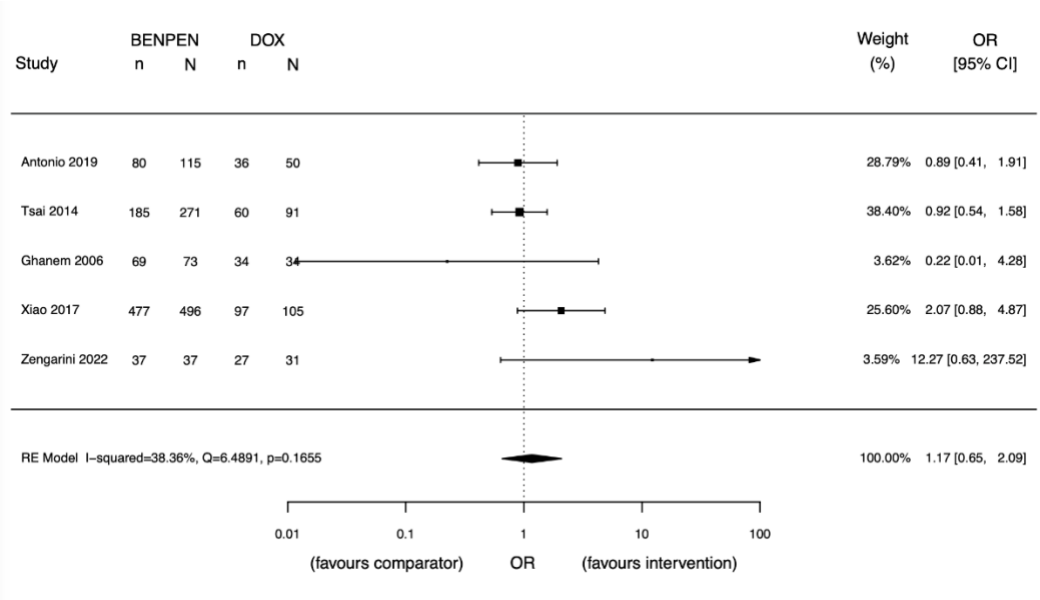
AZM, azithromycin. BENPEN, benzathine penicillin. AMX, amoxicillin. PRO, probenecid. CRO, ceftriaxone. DOX, doxycycline. LNZ, linezolid. PEN, penicillins other than benzathine penicillin.

Note: the line thickness between two nodes is proportional to the inverse variance of the effect estimate for that comparison (i.e., increased line thickness corresponds to narrower credible interval) and node diameter is proportional to the number of participants randomized to that antibiotic agent. Number indicates the number of patients in each node.

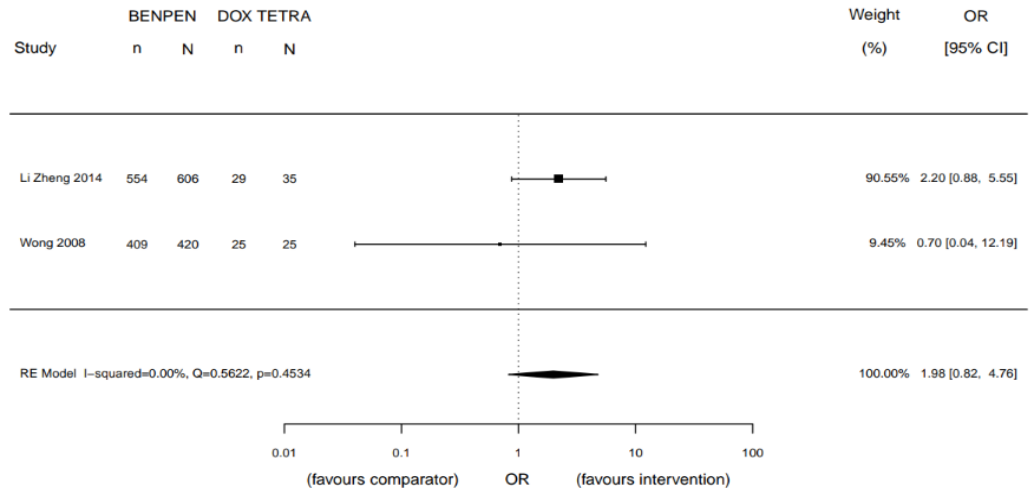
Figure 3. Meta-analyses of observational studies for the primary outcome at 12 month follow up (or nearest time-point).

BENPEN, benzathine penicillin G; DOX, doxycycline; DOX TETRA, doxycycline or tetracycline; TETRA, tetracycline; MIN, minocycline; ERYTHRO, erythromycin; AZITHRO, azithromycin.

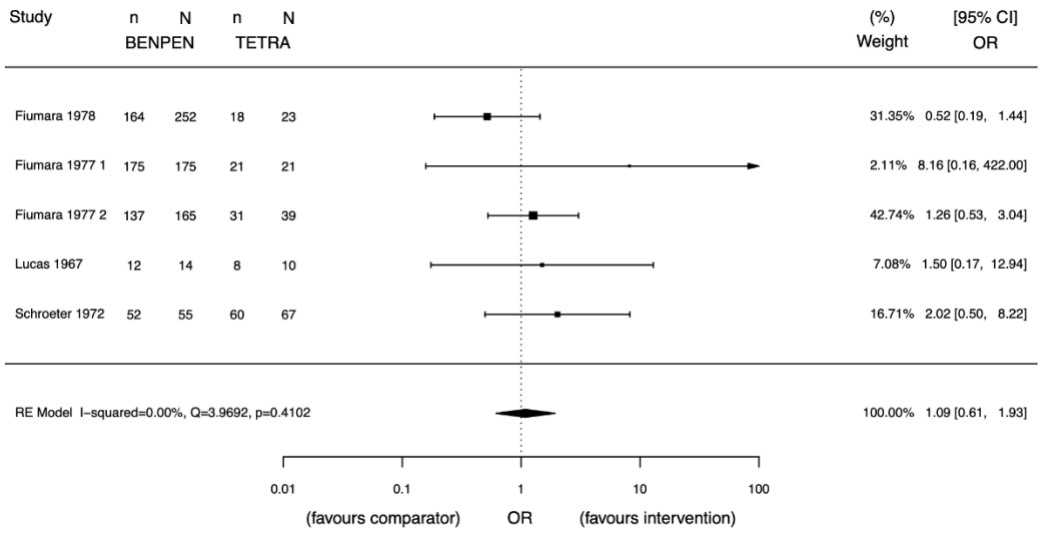
A



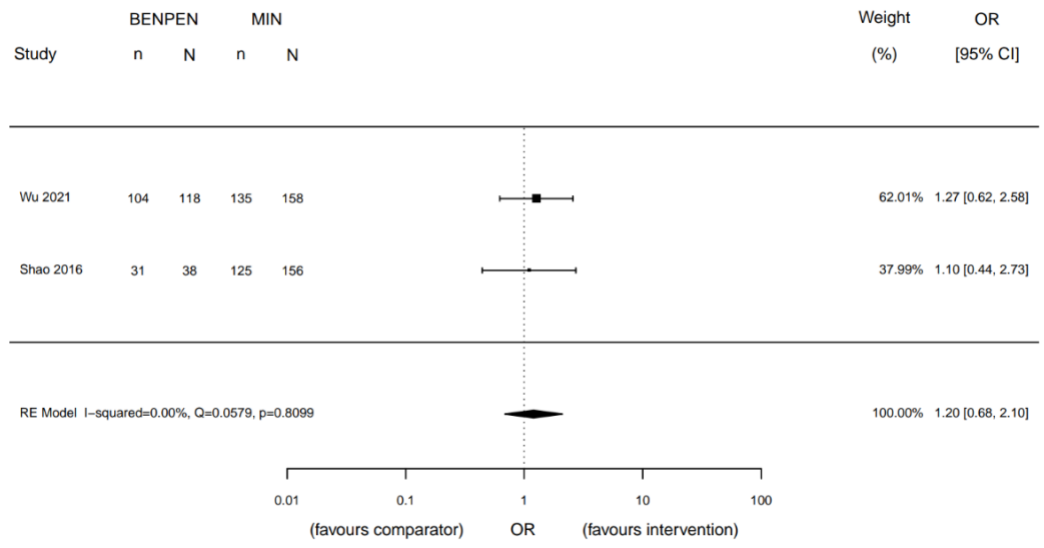
B



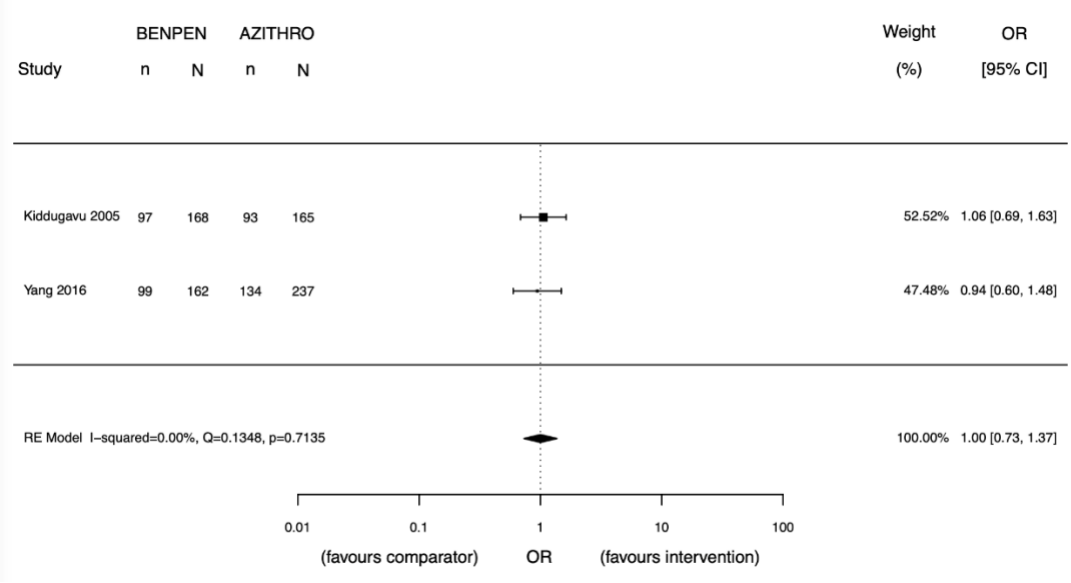
C


Study
n N n N
BENPEN TETRA
(%) Weight
[95% CI] OR
Fiumara 1978 164 252 18 23 31.35% 0.52 [0.19, 1.44]
Fiumara 1977 1 175 175 21 21 2.11% 8.16 [0.16, 422.00]
Fiumara 1977 2 137 165 31 39 42.74% 1.26 [0.53, 3.04]
Lucas 1967 12 14 8 10 7.08% 1.50 [0.17, 12.94]
Schroeter 1972 52 55 60 67 16.71% 2.02 [0.50, 8.22]
RE Model I–squared=0.00%, Q=3.9692, p=0.4102 100.00% 1.09 [0.61, 1.93]
0.01 0.1 1 10 100
(favours comparator) OR (favours intervention)

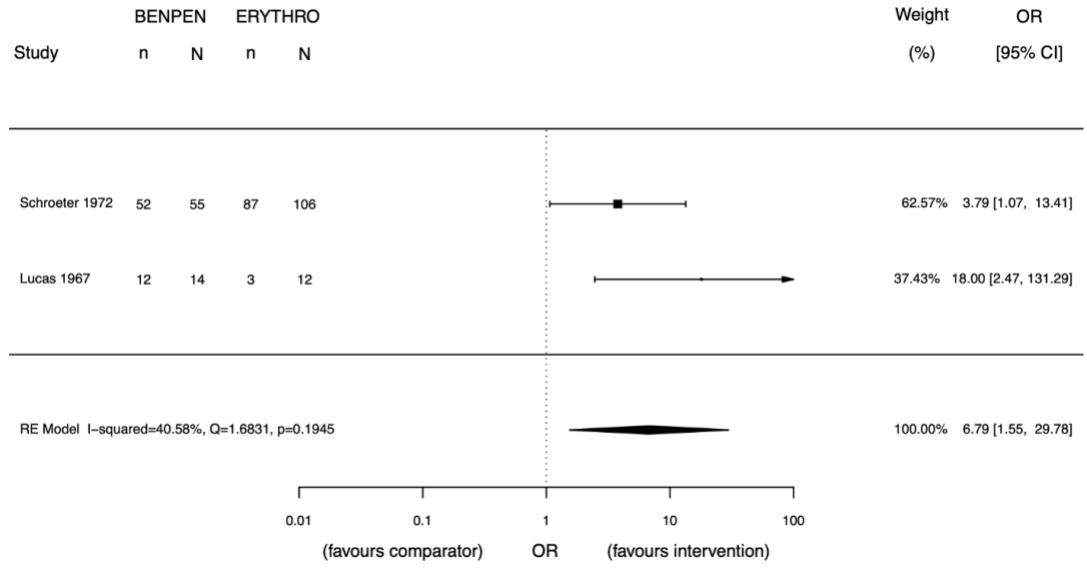
D


BENPEN MIN
Study
n N n N
Weight
(%)
OR
[95% CI]
Wu 2021 104 118 135 158 62.01% 1.27 [0.62, 2.58]
Shao 2016 31 38 125 156 37.99% 1.10 [0.44, 2.73]
RE Model I–squared=0.00%, Q=0.0579, p=0.8099 100.00% 1.20 [0.68, 2.10]
0.01 0.1 1 10 100
(favours comparator) OR (favours intervention)

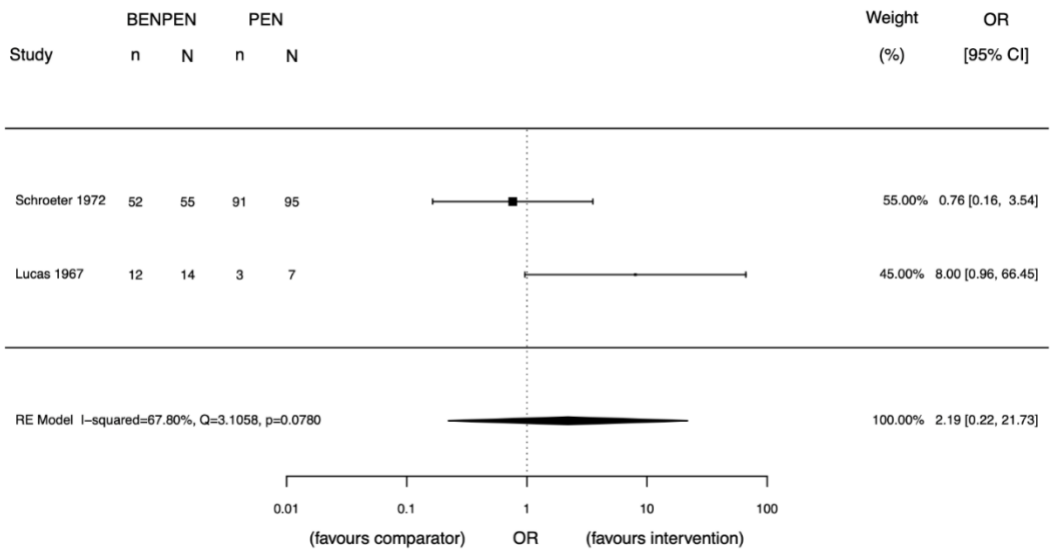
E


BENPEN AZITHRO Weight OR
Study n N n N (%) [95% CI]
Kiddugavu 2005 97 168 93 165 52.52% 1.06 [0.69, 1.63]
Yang 2016 99 162 134 237 47.48% 0.94 [0.60, 1.48]
RE Model I–squared=0.00%, Q=0.1348, p=0.7135 100.00% 1.00 [0.73, 1.37]
0.01 0.1 1 10 100
(favours comparator) OR (favours intervention)

F


BENPEN ERYTHRO Weight OR
Study n N n N (%) [95% CI]
Schroeter 1972 52 55 87 106 62.57% 3.79 [1.07, 13.41]
Lucas 1967 12 14 3 12 37.43% 18.00 [2.47, 131.29]
RE Model I–squared=40.58%, Q=1.6831, p=0.1945 100.00% 6.79 [1.55, 29.78]
0.01 0.1 1 10 100
(favours comparator) OR (favours intervention)

G


BENPEN
PEN
Weight
OR
Study
n
N
n
N
(%)
[95% CI]
Schroeter 1972 52 55 91 95 55.00% 0.76 [0.16, 3.54]
Lucas 1967 12 14 3 7 45.00% 8.00 [0.96, 66.45]
RE Model I–squared=67.80%, Q=3.1058, p=0.0780 100.00% 2.19 [0.22, 21.73]
0.01
0.1
1
10
100
(favours comparator)
OR
(favours intervention)

H

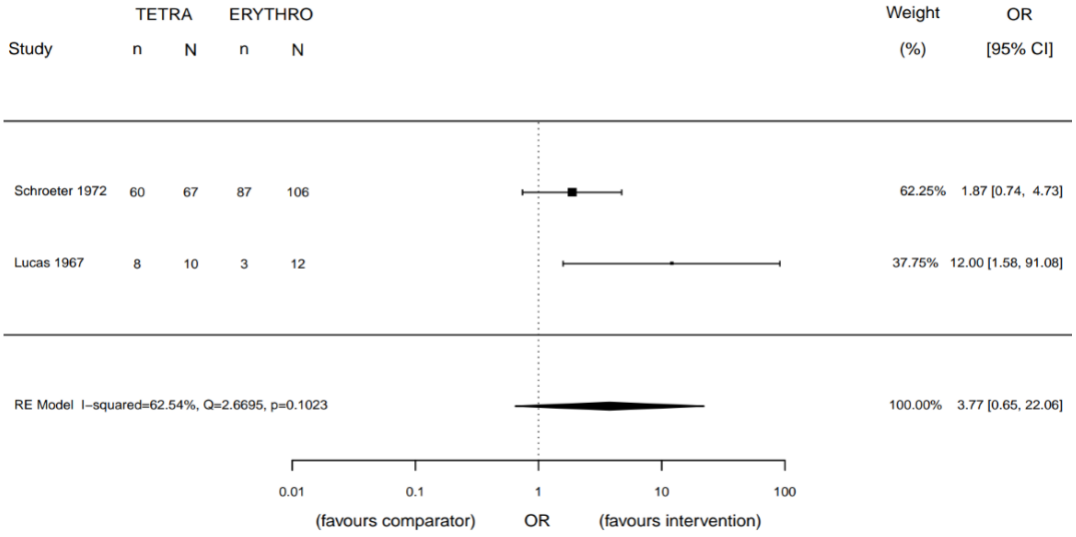

TETRA
ERYTHRO
Weight
OR
Study
n
N
n
N
(%)
[95% CI]
Schroeter 1972 60 67 87 106 62.25% 1.87 [0.74, 4.73]
Lucas 1967 8 10 3 12 37.75% 12.00 [1.58, 91.08]
RE Model I–squared=62.54%, Q=2.6695, p=0.1023 100.00% 3.77 [0.65, 22.06]
0.01
0.1
1
10
100
(favours comparator)
OR
(favours intervention)

Figure 4. Sub-group analysis for people living with HIV of the primary outcome at 12 month follow up (or nearest time-point).

Benzathine penicillin G (BENPEN) versus doxycycline (DOX) among people living with HIV

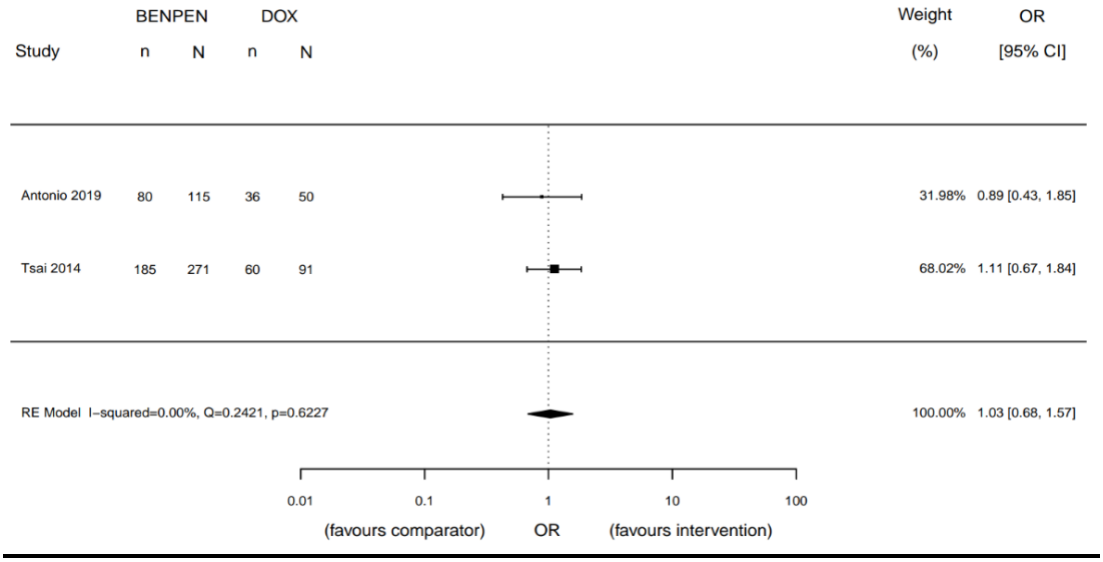
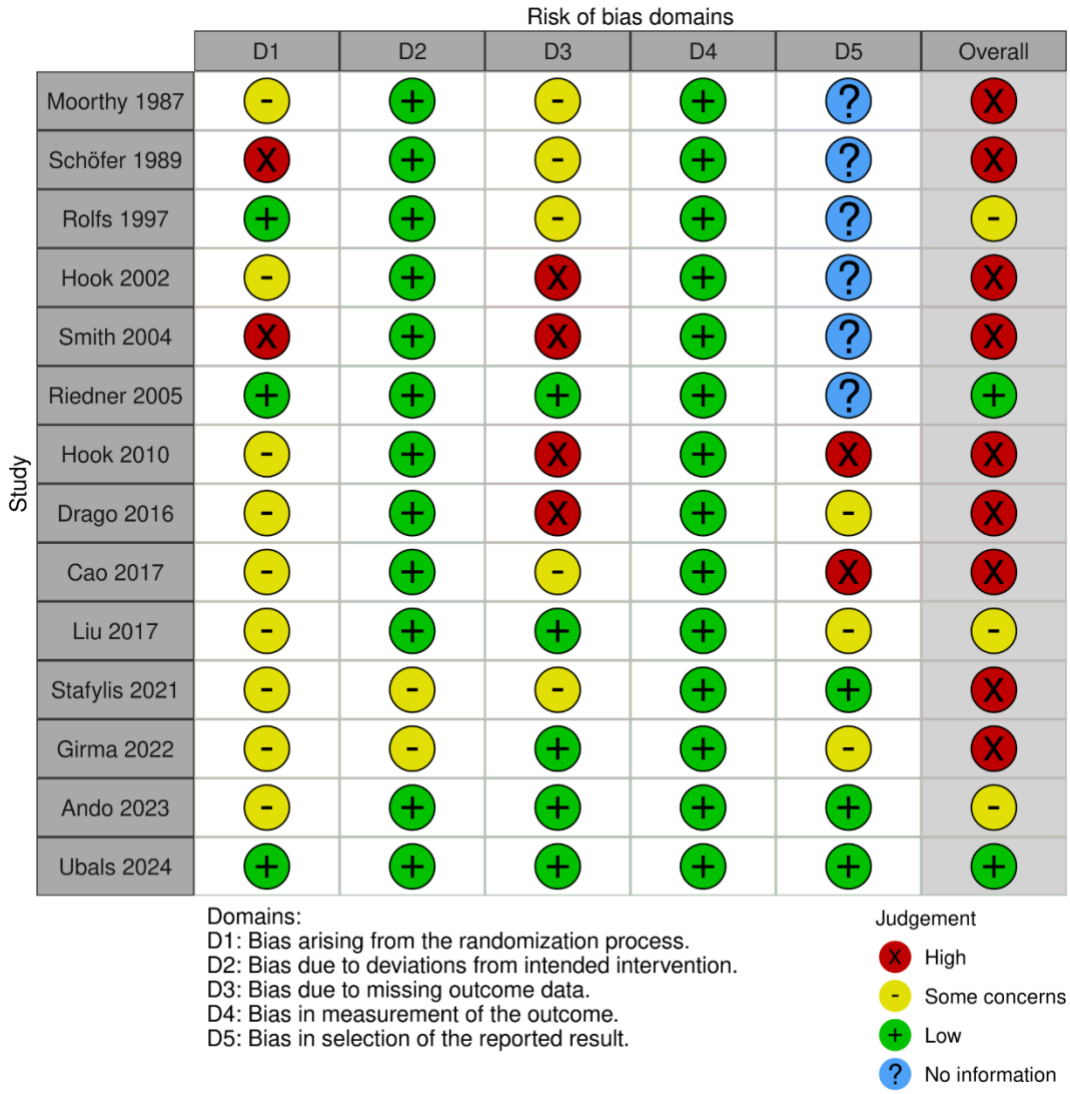
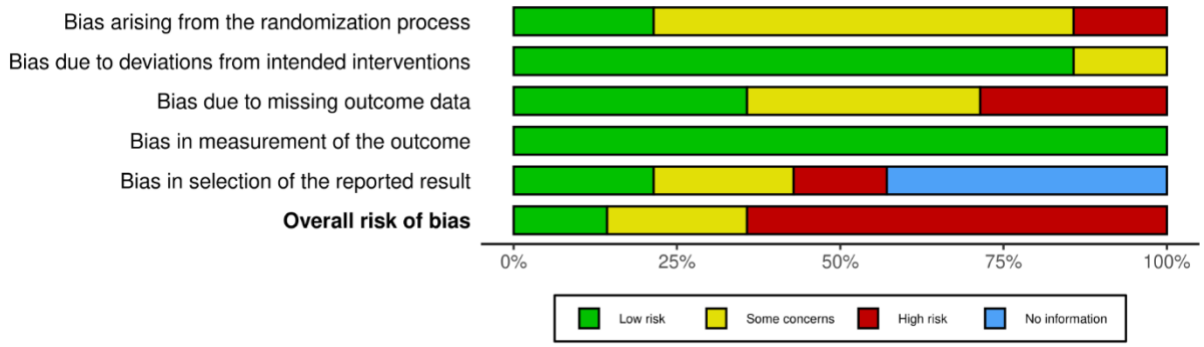


Figure 5. Risk of Bias of RCTs, using the RoB 2 tool. A. Traffic light plot. B. Summary plot.

A



B


Bias arising from the randomization process
Bias due to deviations from intended interventions
Bias due to missing outcome data
Bias in measurement of the outcome
Bias in selection of the reported result
Overall risk of bias
0%
25%
50%
75%
100%
Low risk
Some concerns
High risk
No information

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Appendix 1: Detailed search strategy

#	Query
1	exp "syphilis"/ or "early syphilis".ti,ab,kw. or "lues".ti,ab,kw. or "syphilis".ti,ab,kw. or "syphilitic disorder".ti,ab,kw. or "venereal syphilis".ti,ab,kw.
2	exp "penicillin derivative"/ or "antibiotics, penicillins".mp. or "penicillin".mp. or "penicillin derivate".mp. or "penicillin derivative".mp. or "penicillin series".mp. or "penicillins".mp. or "penicillium extract".mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]
3	exp "ceftriaxone"/ or "7 [2 (2 aminothiazol 4 yl) glyoxylamido] 3 [[(2, 5 dihydro 6 hydroxy 2 methyl 5 oxo 1, 2, 4 triazin 3 yl) thio] methyl] 2 cephem 2 carboxylic acid 7 2 (o methyloxime)".mp. or "acantex".mp. or "axone".mp. or "benaxona".mp. or "biotrakson".mp. or "biotriax".mp. or "bioxon".mp. or "broadced".mp. or "brospec".mp. or "cef-3".mp. or "cefaflox".mp. or "cefalogen".mp. or "cefatriaxone".mp. or "cefaxona".mp. or "cefaxone".mp. or "cefin".mp. or "cefotal".mp. or "cefotriaxon".mp. or "cefotriaxone".mp. or "cefriex".mp. or "ceftrex".mp. or "ceftrian".mp. or "ceftriaxone".mp. or "ceftriaxone and dextrose in duplex container".mp. or "ceftriaxone sodium".mp. or "ceftrilem".mp. or "cefxon".mp. or "ceph 3 em 4 carboxylic acid 7 2 (o methyloxime), 7 [2 (2 aminothiazol 4 yl) glyoxylamido] 3 [[(2, 5 dihydro 6 hydroxy 2 methyl 5 oxo 1, 2, 4 triazin 3yl) thio] methyl]".mp. or "cephin".mp. or "cephtriaxone".mp. or "cerixon".mp. or "cikedrix".mp. or "citeral (ceftriaxone)".mp. or "ecotrixon".mp. or "elpicef".mp. or "eurocef".mp. or "exempla (drug)".mp. or "ferfacef".mp. or "forgram".mp. or "glicocef".mp. or "gomcephin".mp. or "grifotriaxona".mp. or "incephin".mp. or "keftriaxon".mp. or "keptrix".mp. or "lopratin".mp. or "lyceft".mp. or "medoxonum".mp. or "megion".mp. or "mesporin".mp. or "mesporin im".mp. or "mesporin iv".mp. or "monocef".mp. or "nakaxone".mp. or "novosef".mp. or "oframax".mp. or "pantrixon".mp. or "retrokor".mp. or "rinxofay".mp. or "ro 13 9904".mp. or "rocefalin roche".mp. or "rocefin".mp. or "rocephalin".mp. or "rocephin".mp. or "rocephin biochemie".mp. or "rocephin im".mp. or "rocephin im convenience kit".mp. or "rocephin iv".mp. or "rocephin roche".mp. or "rocephin with dextrose".mp. or "rocephine".mp. or "rocephine im".mp. or "rocephine iv".mp. or "rocephine roche".mp. or "rocidar".mp. or "rowecef".mp. or "roxcef".mp. or "roxon".mp. or "samixon".mp. or "sintrex".mp. or "socef".mp. or "sunflow".mp. or "tacex".mp. or "torocef-1".mp. or "trexofin".mp. or "triaken".mp. or "triax".mp. or "triaxone".mp. or "tricefin".mp. or "tricephin".mp. or "trijec".mp. or "xtenda".mp. or "zefaxone".mp. or "zefone 250".mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]
4	exp "erythromycin"/ or "a/t/s".mp. or "abomacetin".mp. or "acneryne".mp. or "acnesol".mp. or "akne mycin".mp. or "akne-mycin".mp. or "aknederm ery gel".mp. or "aknemycin".mp. or "anamycin".mp. or "bonac gel".mp. or "c-solve-2".mp. or "cliniderm".mp. or "deripil".mp. or "duraerythromycin".mp. or "e mycin".mp. or "e-base".mp. or "e-glades".mp. or "e-

	mycin".mp. or "e-solve 2".mp. or "emgel".mp. or "emu v".mp. or "emu-v".mp. or "emu-ve".mp. or "emuvin".mp. or "emycin".mp. or "eriecu".mp. or "erimycin-t".mp. or "eriprodin".mp. or "eritimix".mp. or "eritrex".mp. or "eritrocina".mp. or "eritromicina".mp. or "erixyl".mp. or "ermycin".mp. or "ermysin".mp. or "ery maxin".mp. or "ery-b".mp. or "ery-diolan".mp. or "ery-maxin".mp. or "ery-tab".mp. or "eryacne".mp. or "eryacnen".mp. or "eryc".mp. or "eryc 125".mp. or "eryc ld".mp. or "eryc sprinkles".mp. or "eryc-125".mp. or "eryc-250".mp. or "erycen".mp. or "erycette".mp. or "erycin".mp. or "erycinum".mp. or "eryderm".mp. or "erydermec".mp. or "erydermer".mp. or "eryfluid".mp. or "erygel".mp. or "eryhexal".mp. or "erymax".mp. or "erymaxin".mp. or "erymed".mp. or "erysafe".mp. or "erystrat".mp. or "erytab".mp. or "eryth mycin".mp. or "eryth-mycin".mp. or "erythelan".mp. or "erythmycin".mp. or "erythomycin".mp. or "erythra-derm".mp. or "erythran".mp. or "erythro 200".mp. or "erythro teva".mp. or "erythro-statin".mp. or "erythro-teva".mp. or "erythrogan".mp. or "erythrogel".mp. or "erythrogran".mp. or "erythroguent".mp. or "erythromid".mp. or "erythromycin".mp. or "erythromycin a".mp. or "erythromycin a dihydrate".mp. or "erythromycin base".mp. or "erythromycin base filmtab".mp. or "erythromycin delayed release capsules".mp. or "erythromycine".mp. or "erythromycinum".mp. or "erythroteva".mp. or "erytop".mp. or "erytrarco".mp. or "erytrociclin".mp. or "etinycine".mp. or "etrolate".mp. or "etromycin".mp. or "ilocap".mp. or "ilocaps".mp. or "iloticina".mp. or "ilotycin".mp. or "ilotycin t.s.".mp. or "inderm gel".mp. or "labocne".mp. or "latotryd".mp. or "lederpax".mp. or "mephamycin".mp. or "oftalmolosa cusi eritromicina".mp. or "oftalmolosa cusi erythromycin".mp. or "oftamolets".mp. or "pantodrin".mp. or "pantomycin".mp. or "pantomycin forte".mp. or "pantomycin s 400".mp. or "pce".mp. or "pce dispertab".mp. or "pce dispertabs".mp. or "pharyngocin".mp. or "primacine".mp. or "r-p mycin".mp. or "robimycin".mp. or "romycin".mp. or "roymicin".mp. or "rp mycin".mp. or "rythocin".mp. or "sans-acne".mp. or "sansac".mp. or "skid gel e".mp. or "staticin".mp. or "stiemycin".mp. or "stimycine".mp. or "t stat".mp. or "t-stat".mp. or "theramycin".mp. or "theramycin z".mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]
5	exp "minocycline"/ or "4, 7 bis (dimethylamino) 1, 10, 11, 12a tetrahydroxy 3, 12 dioxo 4a, 5, 5a, 6 tetrahydro 4h tetracene 2 carboxamide".mp. or "4, 7 bis (dimethylamino) 1, 4, 4a, 5, 5a, 6, 11, 12a octahydro 3, 10, 12, 12a tetrahydroxy 1, 11 dioxo 2 naphthacenecarboxamide".mp. or "7 dimethylamino 6 demethyl 6 deoxytetracycline".mp. or "akamin".mp. or "aknemin".mp. or "aknosan".mp. or "amzeeq".mp. or "arestin".mp. or "borymycin".mp. or "cyclimycin".mp. or "cynomycin".mp. or "dynacin".mp. or "klinomycin".mp. or "klinotab".mp. or "lederderm".mp. or "logryx".mp. or "menocycline".mp. or "mestacine".mp. or "micromycin".mp. or "minaxen".mp. or "mino-50".mp. or "mino-wolff".mp. or "minocin".mp. or "minocin g".mp. or "minocin mr".mp. or "minocin pf".mp. or "minoclin".mp. or "minoclir 50".mp. or "minocyclin".mp. or "minocyclin 50 stada".mp. or "minocycline".mp. or "minocycline hydrochloride".mp. or "minocyn".mp. or "minogalen".mp. or "minoline".mp. or "minolira".mp. or "minomax".mp.

	or "minomycin".mp. or "minotab".mp. or "minotab 50".mp. or "minotab-100".mp. or "mirosin".mp. or "mynocine".mp. or "romin".mp. or "skinocyclin".mp. or "solodyn".mp. or "spicline".mp. or "vectran".mp. or "vectrin".mp. or "ximino".mp. or "zilxi".mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]
6	exp "tetracycline"/ or "achromycin".mp. or "achromycin capsules".mp. or "achromycin eye and ear ointment".mp. or "achromycin for oral suspension".mp. or "achromycin intravenous".mp. or "achromycin ointment".mp. or "achromycin ophthalmic oil suspension".mp. or "achromycin ophthalmic sterilized".mp. or "achromycin paediatric drops".mp. or "achromycin pediatric drops".mp. or "achromycin pharyngets".mp. or "achromycin spersoids".mp. or "achromycin surgical powder".mp. or "achromycin syrup".mp. or "achromycin tablets".mp. or "achromycin troches".mp. or "achromycin v".mp. or "achromycin v capsules".mp. or "achromycin v syrup".mp. or "acromicina".mp. or "acromycin".mp. or "actisite".mp. or "agromicina".mp. or "akne pyodron".mp. or "ala tet".mp. or "ala-tet".mp. or "ambamycin".mp. or "ambramicina".mp. or "ambramycin".mp. or "ambrazoo".mp. or "apo tetra".mp. or "apo-tetra".mp. or "apocyclin".mp. or "apotetra".mp. or "artomycin".mp. or "austramycin".mp. or "beatacycline".mp. or "bicycline".mp. or "bio tetra".mp. or "biotetra".mp. or "bitacycline".mp. or "bristaciclina".mp. or "bristacyclin".mp. or "bristacyclina".mp. or "bristacycline".mp. or "bristacycline intravenous".mp. or "bristocycline".mp. or "brodspec".mp. or "cadicycline".mp. or "calociclina".mp. or "cefracycline".mp. or "ciclotetryl".mp. or "clinitetrin".mp. or "clinitetrin syrup".mp. or "combicyclin".mp. or "conmycin".mp. or "copharlan".mp. or "criseociclina".mp. or "cyclabid".mp. or "cyclindif".mp. or "cyclomycetin".mp. or "cyclomycin".mp. or "cyclomycine".mp. or "cyclopar".mp. or "cyclopen".mp. or "deschloraureomycin".mp. or "deschlorbiomycin".mp. or "deschloroaureomycin".mp. or "deschlorobiomycin".mp. or "dhatracin".mp. or "diaciclin".mp. or "dicyclin forte".mp. or "dumocyclin".mp. or "dumocyclina".mp. or "dumocycline".mp. or "economycin".mp. or "enkacyclin".mp. or "epsilontetracycline".mp. or "fabacyclin".mp. or "farciclina".mp. or "fermentmycin".mp. or "fermycine".mp. or "floramicina".mp. or "florocycline".mp. or "hestacyclin".mp. or "hostaciclina".mp. or "hostacyclin".mp. or "hostacycline".mp. or "hostacycline p".mp. or "hostacycline-p".mp. or "hydracycline".mp. or "hydromycin".mp. or "ibicyn".mp. or "ikacycline".mp. or "italcycline".mp. or "kemoclin".mp. or "kristacyclin".mp. or "latycin".mp. or "lenocin".mp. or "mediacycline".mp. or "medicyclin".mp. or "medocycline".mp. or "mervacycline".mp. or "micipan".mp. or "murazine".mp. or "mysteclin".mp. or "mystecline".mp. or "myszeklin".mp. or "neocycline".mp. or "neotetrine".mp. or "novotetra".mp. or "ofticlin".mp. or "omegamycin".mp. or "omnaze".mp. or "orencyclin f 500".mp. or "orencyclin f- 500".mp. or "oricyclin".mp. or "panciclina".mp. or "pancycline wirkstoff".mp. or "panmycin".mp. or "panmycin hydrochloride".mp. or "panmycin p".mp. or "panmycin syrup".mp. or "pansan".mp. or "pantocycline".mp. or "parenciclina".mp. or "pervasol".mp. or "polarcyclin".mp. or "polfamycin".mp. or "polycycline".mp. or "polycycline

	hydrochloride".mp. or "polycycline suspension".mp. or "polycyline".mp. or "polyotic".mp. or "porcycline".mp. or "premocycline".mp. or "purocyclina".mp. or "quadricycline".mp. or "quimocyclar".mp. or "quirvetin".mp. or "recycline".mp. or "remicyclin".mp. or "reseomycin".mp. or "resomicina".mp. or "resteclin".mp. or "retet".mp. or "ricycline".mp. or "rimatet".mp. or "robicyclan".mp. or "robitet".mp. or "sanclomycine".mp. or "sarocycline".mp. or "servitet".mp. or "spaciclina".mp. or "steclin".mp. or "steclin v".mp. or "stilciclina".mp. or "subamycin".mp. or "supramycin".mp. or "tefilin".mp. or "teracyn".mp. or "tesyklin".mp. or "tetra atlantis".mp. or "tetra central".mp. or "tetra wolf".mp. or "tetra-atlantis".mp. or "tetrabakat".mp. or "tetrabid".mp. or "tetrabien".mp. or "tetrabioptal".mp. or "tetrabior".mp. or "tetrablet".mp. or "tetrabon".mp. or "tetrachel".mp. or "tetraciclene".mp. or "tetraciclina".mp. or "tetraciklin".mp. or "tetracitro".mp. or "tetracitro s".mp. or "tetracon".mp. or "tetracyclin".mp. or "tetracycline".mp. or "tetracycline hcl".mp. or "tetracycline hydrochloride".mp. or "tetracycline solution".mp. or "tetracycline syrup buffered".mp. or "tetracyline".mp. or "tetracyn".mp. or "tetracyn capsules".mp. or "tetracyn intravenous".mp. or "tetracyn paediatric drops".mp. or "tetracyn pediatric drops".mp. or "tetracyn syrup".mp. or "tetracyn tablets".mp. or "tetradecin".mp. or "tetrafil".mp. or "tetralen".mp. or "tetralonga".mp. or "tetralution".mp. or "tetramax".mp. or "tetramed".mp. or "tetramig".mp. or "tetrana".mp. or "tetranase".mp. or "tetrano".mp. or "tetraplus".mp. or "tetrarco".mp. or "tetrarco l.a.".mp. or "tetraseptin".mp. or "tetrasuiss".mp. or "tetrecu".mp. or "tetrex paediatric drops".mp. or "tetrex pediatric drops".mp. or "tetrex syrup".mp. or "tetrosol".mp. or "thuricyclin".mp. or "topicycline".mp. or "totomycin".mp. or "triclina".mp. or "triphacyclin".mp. or "tsiklomitsin".mp. or "umetracil".mp. or "veracin".mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]
7	exp "doxycycline"/ or "4 (dimethylamino) 1, 4, 4a, 5, 5a, 6, 11, 12a octahydro 3, 5, 10, 12, 12a pentahydroxy 6 methyl 1, 11 dioxo 2 naphthacenecarboxamide".mp. or "5 hydroxy 6 deoxytetracycline".mp. or "6 deoxy 5 hydroxytetracycline".mp. or "6 deoxy 5 oxytetracycline".mp. or "6 deoxyoxytetracycline".mp. or "6 desoxy 5 hydroxytetracycline".mp. or "6beta deoxyoxytetracycline".mp. or "adoxa".mp. or "alpha 6 desoxy 5 oxytetracycline".mp. or "amermycin".mp. or "atrax".mp. or "azudoxat".mp. or "bactidox".mp. or "banndoclin".mp. or "basedillin".mp. or "bassado".mp. or "biocolyn".mp. or "biodoxi".mp. or "bronmycin".mp. or "calcium doxycycline".mp. or "cloran".mp. or "cyclidox".mp. or "dentistar".mp. or "deoxycycline".mp. or "deoxymycin dispersal".mp. or "deoxymykoin".mp. or "deoxyoxytetracycline".mp. or "desoxy oxytetracycline".mp. or "desoxycycline".mp. or "doinmycin".mp. or "dosil".mp. or "dotur".mp. or "doxaciclin".mp. or "doxacycline".mp. or "doxat".mp. or "doxatet".mp. or "doxi-sergo".mp. or "doxibiotic".mp. or "doxicycline".mp. or "doxilin".mp. or "doximed".mp. or "doximycin".mp. or "doxin".mp. or "doxine".mp. or "doxocycline".mp. or "doxsig".mp. or "doxy".mp. or "doxy 100".mp. or "doxy ii".mp. or "doxy m".mp. or "doxy n tablinen".mp. or "doxy p ratiopharm".mp. or "doxy puren".mp. or "doxy s".mp. or "doxy tablinen".mp. or "doxy-1".mp. or "doxy- caps".mp. or "doxybiocin".mp. or "doxycen".mp. or "doxycen

	retard".mp. or "doxychel".mp. or "doxycin".mp. or "doxycyclin".mp. or "doxycycline".mp. or "doxycycline calcium".mp. or "doxycycline hydrate".mp. or "doxycycline hydrochloride".mp. or "doxycycline monohydrate".mp. or "doxycycline ratiopharm".mp. or "doxycydine monohydrate".mp. or "doxylag".mp. or "doxylin".mp. or "doxymycin".mp. or "doxypuren".mp. or "doxytec".mp. or "doxytrim".mp. or "dumoxin".mp. or "duracycline".mp. or "esdoxin".mp. or "etidoxina".mp. or "gewacyclin".mp. or "gs 3065".mp. or "ibralene".mp. or "idocyclin".mp. or "idocyklin".mp. or "interdoxin".mp. or "investin".mp. or "longamycin".mp. or "lydox".mp. or "magdrin".mp. or "medomycin".mp. or "mespafin".mp. or "mildox".mp. or "miraclin".mp. or "monodox".mp. or "nordox".mp. or "nsc 56228".mp. or "oracea".mp. or "paldomycin".mp. or "pernox gel".mp. or "radox".mp. or "remycin".mp. or "respidox".mp. or "roximycin".mp. or "serodoxy".mp. or "servidoxine".mp. or "servidoxyne".mp. or "siadocin".mp. or "siclidon".mp. or "sigadoxin".mp. or "spanor".mp. or "supracyclin".mp. or "supramycina".mp. or "tenutan".mp. or "tolexine".mp. or "tolexine ge".mp. or "torymycin".mp. or "tsurupioxin".mp. or "unidox".mp. or "veemycin".mp. or "viadoxin".mp. or "vibra s".mp. or "vibra-s".mp. or "vibrabiotic".mp. or "vibracina".mp. or "vibradox".mp. or "vibramicina".mp. or "vibramycin".mp. or "vibramycin calcium".mp. or "vibramycin monohydrate".mp. or "vibramycin n".mp. or "vibramycin-n".mp. or "vibramycine".mp. or "vibraveineuse".mp. or "vibravenos".mp. or "vibravenos sf".mp. or "vibravet".mp. or "viradoxyl-n".mp. or "wanmycin".mp. or "zadorin".mp. or "zenavod".mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kf, fx, dq, bt, nm, ox, px, rx, ui, sy, ux, mx]
8	2 or 3 or 4 or 5 or 6 or 7
9	1 and 8
10	limit 9 to humans
11	limit 10 to yr="2021 -Current"
12	remove duplicates from 11

Supplementary File

Guidance to Assess the Certainty of NMA Evidence

Based on GRADE guidance, we start with high certainty evidence for each estimate. Each GRADE domain is to be rated as "Not serious" (no downgrade), "Serious" (downgrade once) or "Very Serious" (downgrade twice). The final GRADE rating consolidates all of the domains: High (no downgrade in any of the domains), Moderate (downgraded once in total), Low (downgraded twice in total) or Very low (downgraded three or more times in total).

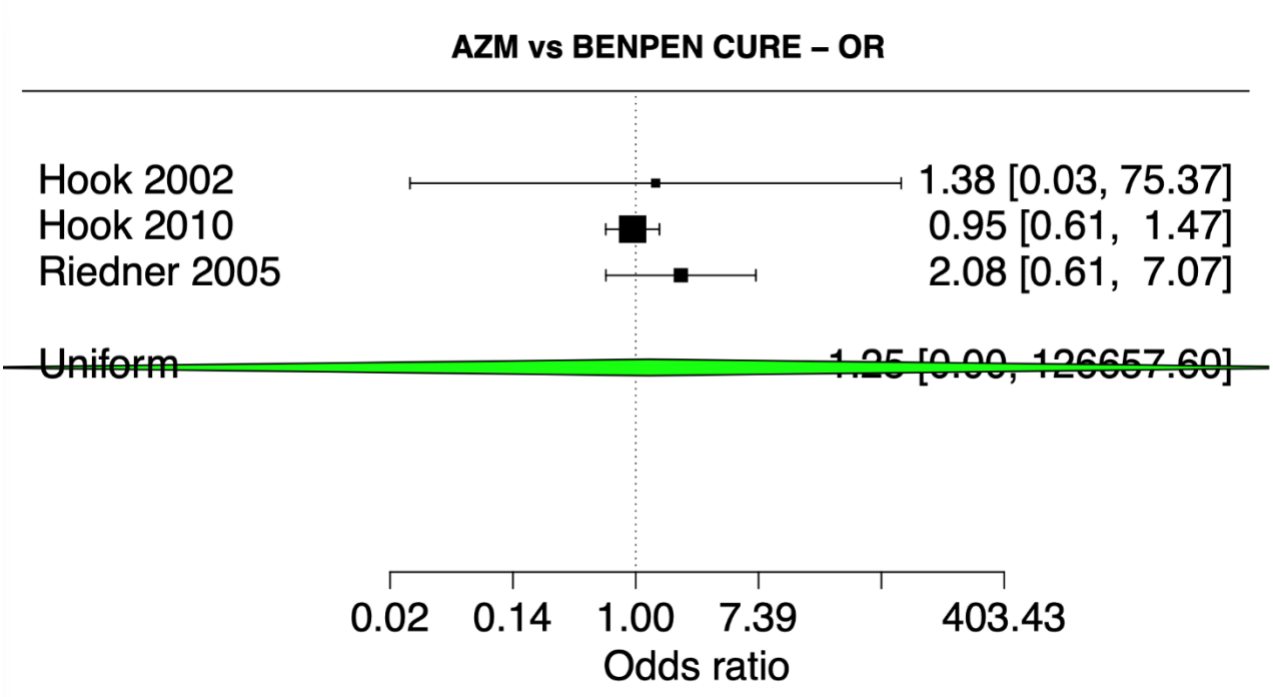
Risk of bias

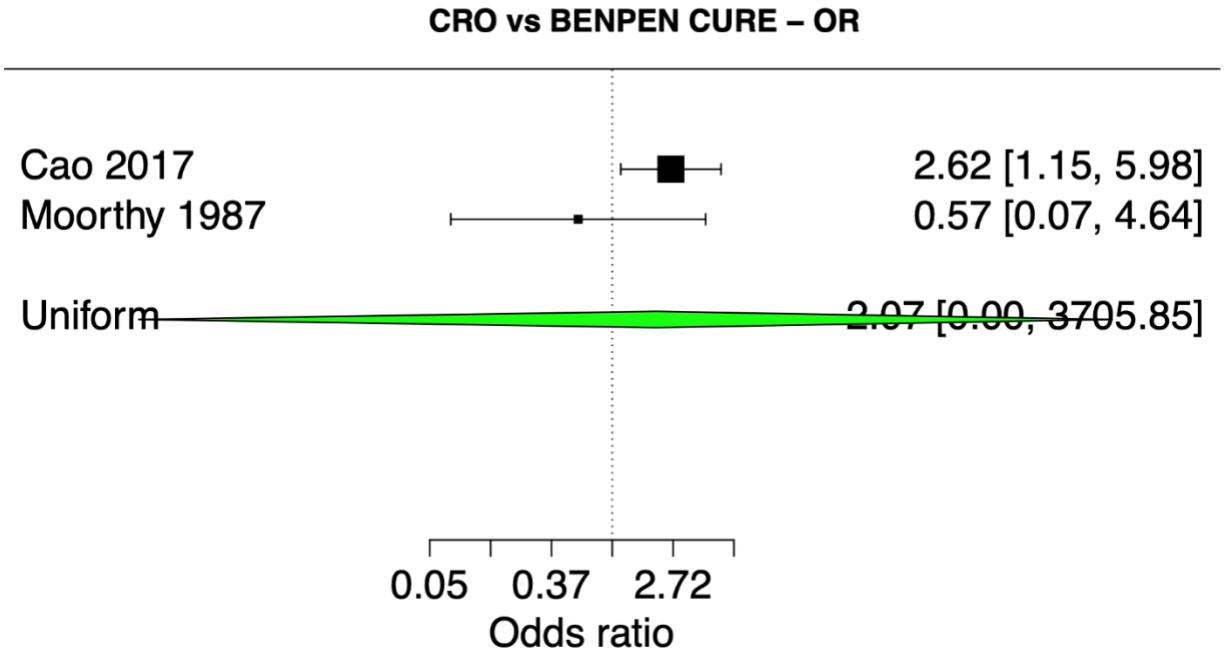
Applies to direct evidence only. Refer to study-level risk of bias. We rated down once for risk of bias if all the studies for that comparison were at high risk of bias overall. We did not rate down for risk of bias more than once.

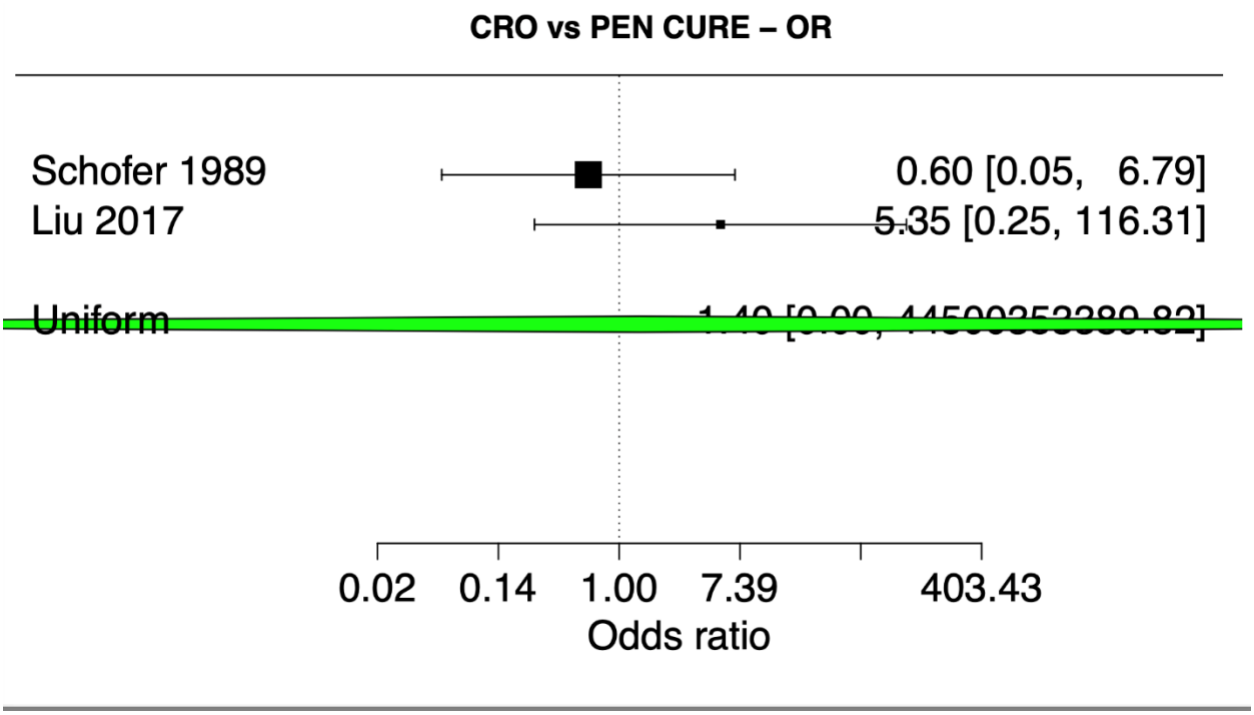
Inconsistency

Applies to direct evidence only where there is at least 2 studies for a given comparison. Assess if forest plots available; otherwise, no serious risk of inconsistency.

Individual study estimates may be inconsistent with each other. We used visual inspection of the forest plots and the I^2 statistic to assess inconsistency. For visual inspection of the forest plots, we considered the variability in point estimates and confidence interval overlap in relation to the null effect. If inconsistency was detected (i.e., confidence intervals did not overlap, presence of considerable (I^2 75-100%) heterogeneity), we rated down once for inconsistency.


AZM vs BENPEN CURE – OR
Hook 2002
Hook 2010
Riedner 2005
Uniform
1.38 [0.03, 75.37]
0.95 [0.61, 1.47]
2.08 [0.61, 7.07]
0.02
0.14
1.00
7.39
403.43
Odds ratio


CRO vs BENPEN CURE – OR
Cao 2017
Moorthy 1987
Uniform
2.62 [1.15, 5.98]
0.57 [0.07, 4.64]
0.05
0.37
2.72
Odds ratio



Imprecision

We downgraded the certainty of the evidence once for imprecision if the 95% CrI of the pooled absolute estimate included the null (based on PHAC's direction) and the CrI included the possibility of important harm (since outcome is cure).

Indirectness

Applies to direct evidence only. We rated down once if the study population, antibiotic route, dose and/or frequency, or the outcome in the primary studies did not accurately reflect the review question. For all direct estimates, we reported no serious risk of indirectness.

Publication bias

Applies to direct evidence only. All estimates had less than 10 RCTs, so we could not assess publication bias by inspecting funnel plots or the Egger's statistical test. We reported no serious risk of publication bias.

Transitivity

The transitivity assumption gauges the appropriateness of combining evidence from individual trials with a common comparator to inform indirect evidence. The distribution of potential effect modifiers should be similar between included trials, otherwise in the presence of large

dissimilarity, rating down for intransitivity may be warranted. We reported no serious risk of intransitivity.

Coherence

Applies only if direct and indirect evidence for the same comparison is available. In the absence of direct and indirect evidence for the same comparison, we report no serious risk of incoherence.

References:

1. Brignardello-Petersen R, Florez ID, Izcovich A, et al. GRADE approach to drawing conclusions from a network meta-analysis using a minimally contextualised framework. Bmj. Nov 11 2020;371:m3900. doi:10.1136/bmj.m3900

2. Guyatt GH, Oxman AD, Vist G, et al. GRADE guidelines: 4. Rating the quality of evidence--study limitations (risk of bias). J Clin Epidemiol. Apr 2011;64(4):407-15. doi:10.1016/j.jclinepi.2010.07.017

3. Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines 6. Rating the quality of evidence--imprecision. J Clin Epidemiol. Dec 2011;64(12):1283-93. doi:10.1016/j.jclinepi.2011.01.012

4. Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines: 8. Rating the quality of evidence--indirectness. J Clin Epidemiol. Dec 2011;64(12):1303-10. doi:10.1016/j.jclinepi.2011.04.014

5. Guyatt GH, Oxman AD, Montori V, et al. GRADE guidelines: 5. Rating the quality of evidence--publication bias. J Clin Epidemiol. Dec 2011;64(12):1277-82. doi:10.1016/j.jclinepi.2011.01.011

6. Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines: 7. Rating the quality of evidence--inconsistency. J Clin Epidemiol. Dec 2011;64(12):1294-302. doi:10.1016/j.jclinepi.2011.03.017

7. Fleischmann M, Vaughan B. Commentary: Statistical significance and clinical significance - A call to consider patient reported outcome measures, effect size, confidence interval and minimal clinically important difference (MCID). J Bodyw Mov Ther. Oct 2019;23(4):690-694. doi:10.1016/j.jbmt.2019.02.009

Guidance to Assess the Certainty of MA Evidence

Based on GRADE guidance for non-randomized studies, we start with low certainty evidence for each meta-analysis. Each GRADE domain is to be rated as "Not serious" (no downgrade), "Serious" (downgrade once) or "Very Serious" (downgrade twice).

Risk of bias

For each meta-analysis, we downgraded the certainty of the evidence once for risk of bias when studies with high risk of bias contributed ≥50% weight to the pooled effect estimates (i.e. serious risk of bias).

Based on the overall NOS score per study, we considered studies with ≥7 stars to be at non-high risk of bias overall and those with ≤6 stars to be at high risk of bias overall.

Inconsistency

We used visual inspection of the forest plots and the I^2 statistic to assess inconsistency. For visual inspection of the forest plots, we considered the variability in point estimates and confidence interval overlap in relation to the null effect. Further, we downgraded the certainty of the evidence once when there was considerable (I^2 75-100%) heterogeneity.

Imprecision

We downgraded the certainty of the evidence once for imprecision if the 95% CI of the pooled absolute estimate included the null (based on PHAC's direction) and the CI included the possibility of important harm (since outcome is cure). The threshold for harm utilized was a non-inferiority margin of 15%, which is standard from much of the Infectious Diseases literature.

Indirectness

We rated down once for indirectness if the study sample in the primary studies did not accurately reflect the review question or population of interest. We did this when studies which included a mix of early and late syphilis patients which could not be categorized into either early- or late-stage syphilis categories, contributed >30% weight to the pooled effect estimates, and in comparisons done in populations with resistance patterns which do not match current resistance patterns in our population of interest.

Publication bias

All estimates had less than 10 RCTs, so we could not assess publication bias by inspecting funnel plots or the Egger's statistical test. We reported no serious risk of publication bias.

NMA subgroup analysis for early syphilis