

SYSTEMATIC REVIEW

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Research mapping and comparative assessment of drug treatment regimens for brucellosis: a systematic review and network meta-analysis

Abbas Pele¹, Sima Masudi¹, Rahim Nejad Rahim² and Mohammad Heidari^{1*}

Abstract

Objective There are different treatment regimens for treating human brucellosis. This study aimed to evaluate the comparative effectiveness and ranking of different treatment regimens for human brucellosis through a systematic review and network meta-analysis (NMA).

Methods A systematic search was conducted in PubMed, ScienceDirect, Web of Science, and Embase to identify randomized controlled trials (RCTs) from their inception until February 1, 2023. Only RCTs comparing different treatment regimens for human brucellosis were included. The risk of bias was assessed using the Cochrane Risk of Bias tool. Consistency and heterogeneity were evaluated using Cochrane's I^2 and Q statistics. Our outcomes of interest include the "recovery" of patients with brucellosis following pharmacological treatment, as well as the "relative ranking" of the effectiveness of the studied treatment regimens compared to one another. Treatment effects were estimated using the relative risk (RR) effect size, while the P-score and Sucre was utilized to rank treatments in the NMA. A random-effects model was applied to account for between-study variability, and results were reported with 95% confidence intervals (CI).

Results Out of 872 primary studies reviewed, a total of 32 studies were included in our network meta-analysis. The comparative effectiveness (measured by improvement) of the doxycycline (DOX) + streptomycin (STR) + hydroxychloroquine (HYD) treatment regimen (RR:1.25, 95%CI: 1.07–1.45) is greater than other treatment regimens compared to DOX + rifampin (RIF) (RR:1). Following that, the DOX + STR + RIF and STR + ofloxacin (OFL) + RIF treatment regimens ranked second and third, respectively. The heterogeneity of the designs in our intervention network was estimated to be 37%, indicating low inconsistency within an acceptable range.

Conclusion The findings highlight the superior efficacy of triple-drug regimens, particularly those involving aminoglycosides, over dual-drug and monotherapy options. Our results confirm that DOX + STR + HYD is the most effective treatment regimens for human brucellosis. These new findings, when considering practical considerations, could inform the updating of clinical treatment protocols for human brucellosis.

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Keywords Systematic review, Network meta-analysis, Brucellosis, Treatment

Background

Brucellosis is the most prevalent zoonotic disease globally, posing a significant health challenge in several countries, including Iran [1–4]. Despite the availability of effective antibiotics for over half a century, its treatment remains problematic, with approximately 500,000 cases reported annually—only a fraction of the actual incidence [2]. The World Health Organization (WHO) recommended a six-week doxycycline-rifampin regimen in 1986, yet studies have linked this approach to relapses and treatment failures [5–7]. Treatment regimens and durations continue to vary across medical centers [1]. Given the limitations of existing therapies, including side effects, relapses, and failures, a reassessment of both established and novel drug options is essential to optimizing treatment outcomes [8]. Combination therapy remains the standard approach, as monotherapy is associated with relapse rates of up to 30% [2, 9].

Various drug regimens, both combined and single, have been introduced for the treatment of brucellosis. These include combinations such as rifampin(RIF)+doxycycline(DOX), doxycycline+rifampin+streptomycin(STR), doxycycline+gentamicin(GEN), doxycycline+streptomycin, and tetracycline(TET)+streptomycin, as well as several other drug combinations. Single drug regimens, such as rifampin, cotrimoxazole(trimethoprim/sulfamethoxazole (TMP/SMX))(COT), doxycycline, and ciprofloxacin(CIP), have also been used but are now largely restricted or eliminated after years of use [10]. Several systematic reviews and meta-analyses have been conducted to evaluate the efficacy of various antibiotic regimens for human brucellosis. For instance, Skalsky et al. (2008) performed a systematic review and meta-analysis of randomized controlled trials, concluding that prolonged treatment with dual or triple regimens, including an aminoglycoside, is more effective in reducing relapse rates compared to monotherapy [11]. Similarly, Solís García del Pozo and Solera (2012) conducted a meta-analysis of randomized clinical trials (RCTs), highlighting the superiority of combination therapies over single-drug treatments in achieving better clinical outcome [12]. Despite the availability of various treatment regimens for human brucellosis, their relative effectiveness and ranking remain unclear. This means to date, no network meta-analysis has systematically synthesized evidence from RCTs to assess and rank the relative effectiveness of all treatment strategies for human brucellosis. This gap underscores the need for a comprehensive analysis to inform clinical decision-making and optimize treatment protocols. RCTs provide the highest level of evidence for evaluating therapeutic interventions; however, no single

RCT has compared all available treatments simultaneously. Conventional meta-analyses are limited to pairwise comparisons, restricting their ability to comprehensively assess multiple interventions. Network meta-analysis (NMA) addresses this limitation by integrating direct and indirect evidence, allowing for the simultaneous comparison and ranking of multiple treatment options [13, 14].

Given that brucellosis treatment regimens have not yet been evaluated using NMA, this study aims to systematically assess and rank available therapeutic interventions based on their effectiveness.

Methods

The protocol for this systematic review and meta-analysis has been registered in PROSPERO (ID: CRD42021291074) and approved by the Ethical Committee of Urmia University of Medical Sciences (Approval No: IR.UMSU.HIMAM.REC.1401.061).

Literature search

PubMed, ScienceDirect, Web of Science, Embase, ProQuest, Scopus were searched for RCTs comparing the effectiveness of brucellosis treatments published between inception and Feb 1 2023. We employed a comprehensive search strategy using terms related to brucellosis, randomized controlled trials (RCTs), and antibiotics, both individually and in combination (Table S1). To ensure that no grey literature was overlooked, the author searched Google and publicly available thesis databases for potential primary studies that might have been missed in the main study databases. Ultimately, it was confirmed that all relevant articles were already listed in the main study databases, and no additional findings were identified through Google searches. Screening was conducted by two independent authors, and any discrepancies between their findings were resolved through discussion and consensus. A search strategy was developed based on the PICOS framework: Population (P): those patients diagnosed with human Brucellosis; Interventions (I): antibiotics; Comparisons (C): an antibiotic versus alternative antibiotic; Outcomes (O): recovery; and Study design (S): RCTs. All details of the study inclusion and exclusion criteria are provided in the Table S2.

Inclusion and exclusion criteria

The study includes patients diagnosed with human brucellosis, confirmed through serological (Wright $\geq 1/160$, 2-Mercaptoethanol $\geq 1/80$) or culture-based methods, considering clinical findings and associated risk factors (Table S2). Both sexes and all age groups are eligible for inclusion. Interventions of interest comprise antibiotic or

chemical drug regimens evaluated in RCTs, with at least one direct comparison reported in a primary study. The reference comparator in this study is the World Health Organization-recommended combination of doxycycline and rifampin (DOX + RIF). However, this selection is a matter of convention, as any of the evaluated treatment regimens could have been designated as the comparator group. Eligible study designs include randomized controlled trials (RCTs) published in English or in other languages such as Spanish, French, German, Chinese, or Persian, provided that full-text translation into English is reliably achievable, with availability in reputable, indexed journals. The exclusion criteria encompass studies on non-human brucellosis, patients with co-infections or severe comorbidities that could confound treatment outcomes, non-standard or alternative therapies, observational studies, systematic reviews, meta-analyses, in vitro or animal research, single-arm clinical trials, studies lacking sufficient data for direct or indirect comparisons, and those with a high risk of bias, as assessed using tools such as the Cochrane Risk of Bias tool.

Data extraction and management

All primary studies that met the inclusion and exclusion criteria were imported into EndNote software (version 20.4.01). The data were extracted by the first author of the article (Abbas Pele) and then reviewed by the corresponding author (Dr. Mohammad Heidari). The extracted data from the included studies encompassed the following variables: the name of the corresponding author, year of publication, country where the study was conducted, number of participants in each trial arm, gender distribution in each arm, mean age of participants in each arm, type of treatment regimen administered in each study arm, drug dosage or combination, and duration of drug administration. Additionally, outcome-related data included the number of patients in each study arm categorized by recovery, treatment failure, relapse, and a combined outcome of treatment failure and relapse, representing overall treatment failure.

Statistical methods

In this study, the primary outcome was treatment success, defined as clinical remission in patients with human brucellosis, while secondary outcomes were not considered in this study. In this study, to analyze the primary studies and desired outcomes, we used the relative risk (RR) effect size with a 95% confidence interval to estimate the final comparison of study arms. Meta analysis was performed using the frequentist approach and random-effects model with a 95% confidence interval. All analysis were conducted using R version 4.3.0 (packages netmeta, meta,

remote, dmetar, MASS, abind, magic, ggplot2, metafor, tidyverse, and rgl) and STATA version 18 (command mvmeta). For the final ranking of treatments using the classical method, each treatment was ranked using the P-score and Sucra. P-scores and Forest plot diagrams were used to rank and display the relative effect of drugs. This network meta-analysis was conducted and reported in accordance with the PRISMA-NMA guidelines. Relevant names of drug compounds used were abbreviated, using the first three letters of each drug's name (Table S3).

Evaluation of the quality and risk of bias of the studies

To evaluate the quality of the selected studies, we used the standard Cochrane Risk of Bias (RoB) tool in RevMan software (version 5.4.1). After assessing the quality of the selected trials across seven domains, the studies were classified into three categories: low risk of bias, high risk of bias, and unclear risk of bias, based on the scores obtained. The transitivity assumption suggests that the studies included in the network are sufficiently similar in terms of clinical and methodological characteristics, allowing indirect comparisons to be used for drawing conclusions. To verify the assumptions of the NMA, the selected studies were reviewed by the researchers and scientific advisors based on the requirements for combining direct and indirect comparisons within the network. The clinical similarity between studies was assessed by examining patient characteristics (age, gender, disease severity, history of prior treatments), treatment interventions (dose, treatment duration, administration method), and outcomes (assessment of recovery, treatment failure, relapse).

Assessment of heterogeneity

Heterogeneity was evaluated using Cochran's Q test, τ^2 (tau-squared), and I^2 statistics. The heterogeneity within direct comparisons was quantified using the τ^2 (tau-squared) and I^2 statistics. I^2 values of 25%, 50%, and 75% were interpreted as low, moderate, and high heterogeneity, respectively. The I^2 statistic was also used to determine whether a fixed-effects or random-effects model should be applied. Additionally, we assessed the inconsistency or heterogeneity of our study at three levels: total heterogeneity within the network, within-design heterogeneity, and between-study heterogeneity, and we statistically analyzed its significance. To locate inconsistency within the network using the "Net Heat Plot" diagram, we applied the internal function "nethefiat" in the R software environment. To Assessment of Publication Bias we employed Comparison-Adjusted Funnel Plot: Funnel plots were visually inspected, and asymmetry was tested using Egger's test.

Results

Process and results of literature retrieval

From a total of 872 articles identified from our search (Fig. 1), 32 were included in our study (Table 1). The primary studies included a total of 3,670 participants, all of whom were malaria patients treated with at least one of the study arms. The search strategy used in this study is outlined in Table S1, while Table S2 categorizes the criteria for inclusion and exclusion of network meta-analysis. The treatments analyzed in this network meta-analysis included: doxycycline, streptomycin, ciprofloxacin, ofloxacin (OFL), cotrimoxazole, ceftriaxone (CEF), trimethoprim (TRI), tetracycline, rifampicin/rifampin, sulfamethoxazole (SUL), gentamicin, hydroxychloroquine (HYD), levofloxacin (LEV), amikacin (AMI) (Table S3).

Basic characteristics and quality evaluation of the included literature

The analysis of the selected studies identified 22 unique drug regimens. In total, 3,670 patients with brucellosis were included across 32 studies and were subjected

to various treatment protocols. The risk of bias assessment, conducted using the standard Cochrane Risk of Bias (RoB) tool, is presented in Fig. 2 and Figure S1. The assumption of similarity in clinical and epidemiological characteristics was met for all RCTs included in this review.

Evidence network

As shown in Fig. 3, both direct and indirect evidence exists among the interventions (drug treatment regimens for brucellosis). The network contains 12 closed loops (triangular or higher-order cycles), which facilitate multiple pathways for indirect comparisons, thereby strengthening the robustness of the analysis. Furthermore, there are 32 direct pairwise comparisons, as indicated by the connecting edges, reflecting the number of studies that directly compared two treatment interventions. This well-connected network structure ensures a comprehensive evaluation of the relative effectiveness of various treatment options for brucellosis.

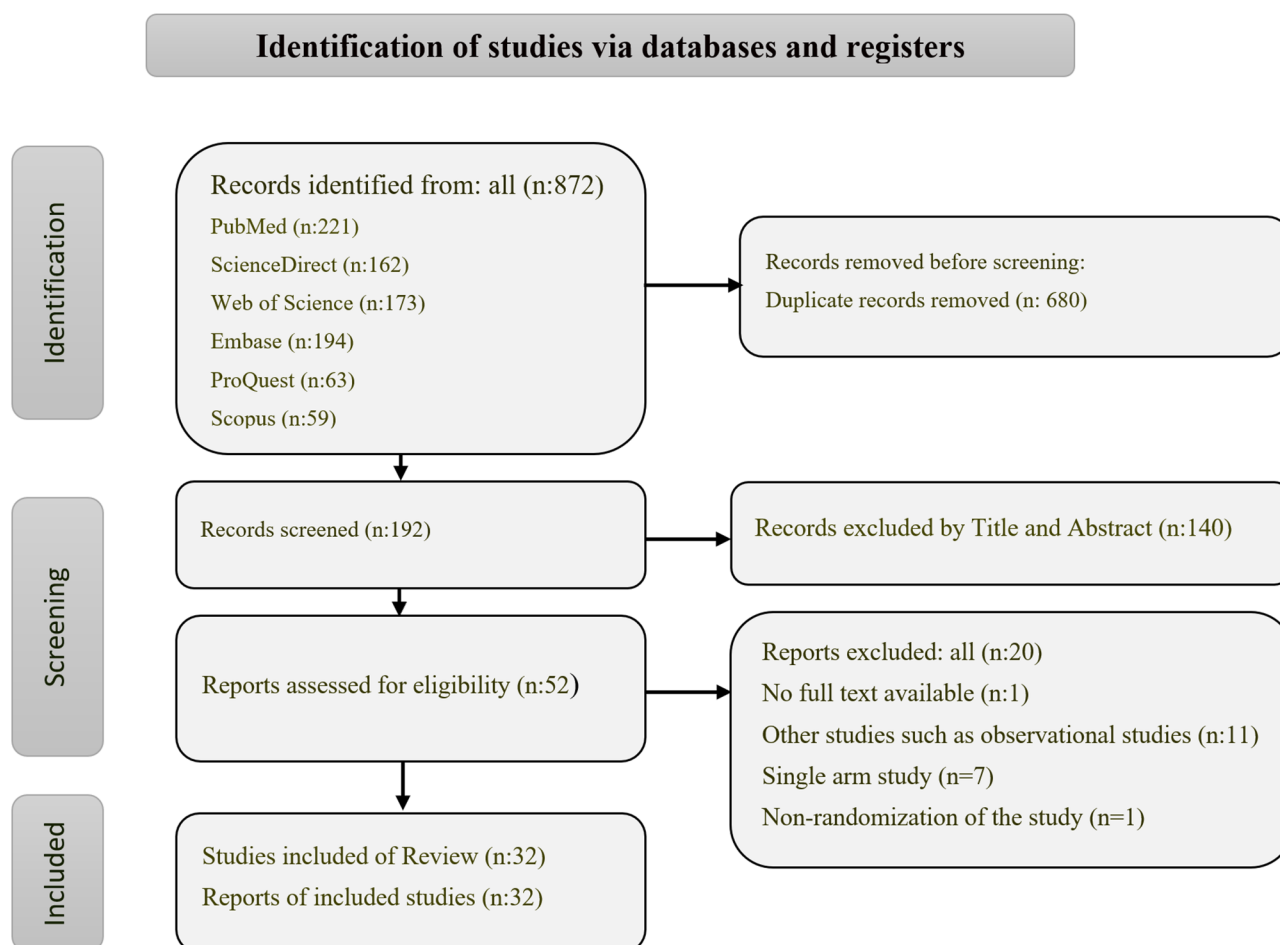


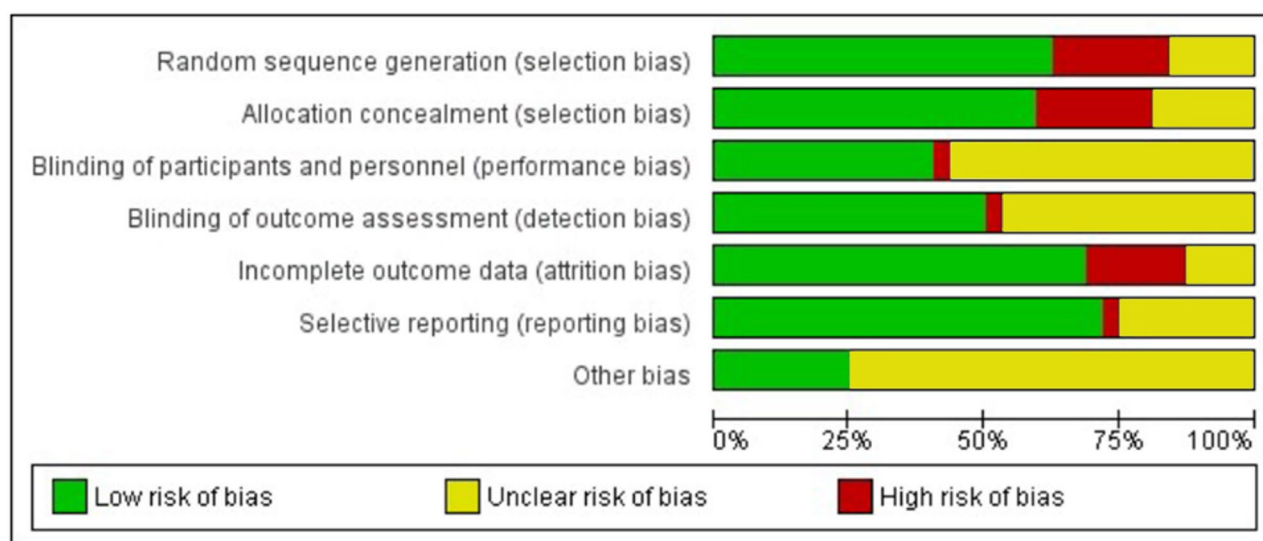
Fig. 1 Flowchart of search and selection of articles for study

Table 1 List of final selected RCT studies

Study - Name of the first author	year of publication	Country	Number of study arms	Therapeutic regimens	Total number of participants	Proportion of Male gender in total participants	Average age of all participants
Alavi [15]	2007	Iran	2	DOX + RIF	51	-	30
				DOX + COT	51	-	31
Acocella [16]	1989	France, Greece, Spain	3	DOX + RIF	63	57%	39
				DOX + STR	53	77%	40
				STR + TET	27	46%	45
Agalar [17]	1999	Turkey	2	DOX + RIF	20	65%	37
				CIP + RIF	20	40%	38
Akova [18]	1993	Turkey	2	OFL + RIF	31	45%	38
				DOX + RIF	30	53%	34
Alikhani [19]	2007	Iran	2	DOX + STR + RIF	37	-	-
				STR + OFL + RIF	38	-	-
Alp [20]	2006	Turkey	2	DOX + STR	15	60%	48
				CIP + RIF	16	50%	68
Ariza [21]	1985	Spain	2	COT	28	89%	32
				STR + TET	27	74%	33
Ariza [22]	1992	Spain	2	DOX + RIF	44	70%	37
				DOX + STR	51	72%	42
Bayindir [23]	2003	Turkey	5	STR + TET	20	60%	45
				DOX + STR	21	52%	39
				DOX + STR + RIF	22	54%	42
				DOX + RIF	20	55%	41
				OFL + RIF	19	53%	37
Colmenero [24]	1989	Spain	2	DOX + STR	59	76%	33
				DOX + RIF	52	61%	33
Colmenero [25]	1994	Spain	2	DOX + STR	10	70%	35
				DOX + RIF	10	60%	32
Didgar [26]	2014	Iran	2	DOX + RIF	39	71%	39
				DOX + CIP	41	80%	36
Ersoy [27]	2005	Turkey	3	OFL + RIF	41	56%	35
				DOX + RIF	45	53%	37
				DOX + STR	32	47%	37
Hasanain [28]	2016	Egypt	2	DOX + RIF	53	60%	33
				DOX + RIF + LEV	54	56%	36
Hashemi [29]	2012	Iran	3	OFL + RIF	64	56%	41
				DOX + RIF	62	55%	39
				DOX + STR	65	55%	40
Kalo [30]	1996	Tirana	2	DOX + CIP	12	58%	32
				DOX + RIF	12	58%	32
Karabay [31]	2004	Turkey	2	DOX + RIF	14	79%	29
				OFL + RIF	15	87%	35
Keramat [32]	2009	Iran	3	CIP + RIF	62	55%	44
				DOX + CIP	55	51%	38
				DOX + RIF	61	71%	40
Lang [33]	1990	Israel	2	DOX + RIF	4	25%	32
				CIP	6	67%	43
Lang [34]	1992	Israel	2	CEF	8	13%	26
				DOX + STR	10	40%	30
Majzoobi [35]	2018	Iran	2	DOX + STR	88	66%	43
				DOX + STR + HYD	89	66%	39
Mile [36]	2012	Macedonia	2	DOX + RIF	94	77%	33
				DOX + RIF + GEN	87	72%	33

Table 1 (continued)

Study - Name of the first author	year of publication	Country	Number of study arms	Therapeutic regimens	Total number of participants	Proportion of Male gender in total participants	Average age of all participants
Montejo [37]	1993	Spain	4	DOX + RIF	111	77%	46
				TRI + SUL	64	66%	46
				DOX	71	76%	46
				DOX + STR	84	73%	46
Ranjbar [38]	2007	Iran	2	DOX + RIF + AMI	110	49%	36
				DOX + RIF	110	48%	37
Roushan [39]	2004	Iran	2	DOX + COT	140	53%	36
				COT + RIF	140	54%	31
Roushan [40]	2006	Iran	2	DOX + GEN	97	59%	34
				DOX + STR	94	55%	36
Roushan [41]	2010	Iran	2	DOX + GEN	82	68%	36
				DOX + STR	82	63%	37
Saltoglu [42]	2002	Turkey	2	DOX + RIF	30	27%	37
				OFL + RIF	27	22%	37
Sarmadian [43]	2014	Iran	2	DOX + CIP	39	79%	39
				DOX + RIF	41	71%	36
Shehabi [44]	1990	Jordan	4	STR + TET	56	50%	27
				TET + RIF	24	50%	27
				COT + RIF	16	50%	27
				RIF	10	50%	27
Solera [45]	1995	Spain	2	DOX + RIF	100	80%	33
				DOX + STR	94	84%	34
Hassan [46]	2022	Egypt	2	DOX + COT	50	74	39
				DOX + RIF	50	68	38

**Fig. 2** The size of the total estimated risk of bias of the studies based on the type of bias**Network meta-analysis**

The DOX + RIF regimen was designated as the reference treatment arm for comparison with alternative therapeutic strategies, and the relative risk (RR) effect size of the remaining treatment arms was evaluated against

this reference using a random-effects model. The league table (Table S6) presents pairwise comparisons of 22 different treatment regimens for human brucellosis, analyzed through network meta-analysis (NMA). This table enables a comprehensive evaluation of both direct and

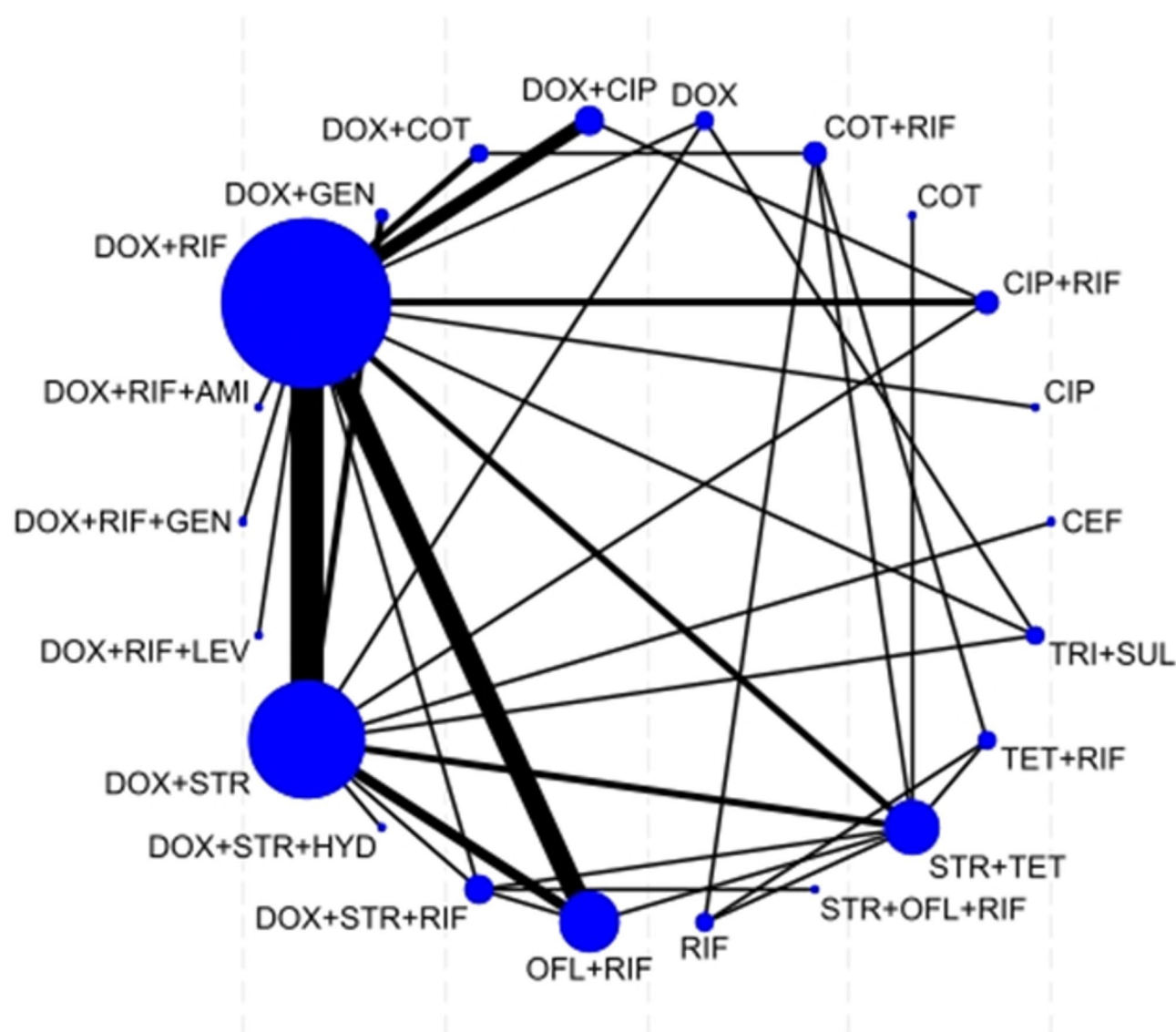


Fig. 3 Network diagrams of brucellosis treatment interventions

combined direct-indirect comparisons, facilitating the identification of the most effective treatment strategies based on RR estimates. Each cell within the matrix represents a two-way comparison, where RR values indicate the relative superiority or inferiority of one treatment over another. The findings indicate that combination therapies incorporating doxycycline (DOX) alongside rifampin (RIF) and aminoglycosides (e.g., gentamicin (GEN) and streptomycin (STR)) consistently demonstrate superior efficacy compared to monotherapies, including ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (TRI+SUL), rifampin alone, and cephalosporins (CEF). For instance, the DOX+RIF+GEN regimen exhibited RR values below 1 in most pairwise comparisons, suggesting a lower risk of treatment failure relative to other

regimens. Additionally, the DOX+STR+RIF combination emerged as one of the most effective therapeutic options, displaying a significantly lower RR compared to less intensive regimens such as DOX+RIF or COT+RIF, thereby indicating enhanced efficacy in preventing treatment failure. In contrast, monotherapies and certain lower-intensity combination therapies, including CIP, CEF, and TRI+SUL, were associated with higher RR values, suggesting a greater likelihood of treatment failure compared to more potent regimens. Collectively, the results from the league table indicate that combination therapies, particularly those incorporating doxycycline, rifampin, and aminoglycosides, provide superior efficacy compared to alternative regimens.

The forest plot (Fig. 4) further illustrates the comparative effectiveness of various treatment regimens for human brucellosis, with DOX+RIF serving as the reference treatment. The plot presents RR estimates along with corresponding 95% confidence intervals (CIs) for each regimen, allowing for a visual assessment of treatment efficacy. The findings indicate that CEF (cephalosporins) and CIP (ciprofloxacin) exhibit significantly

lower RR values (0.15 [0.02, 0.96] and 0.22 [0.03, 1.46], respectively), suggesting markedly reduced effectiveness in comparison to DOX+RIF. Conversely, most combination therapies, particularly those including aminoglycosides such as STR or GEN, yielded RR values above 1, implying a potential increase in treatment efficacy, albeit with overlapping confidence intervals. Notably, the DOX+STR+HYD (hydrocortisone) regimen

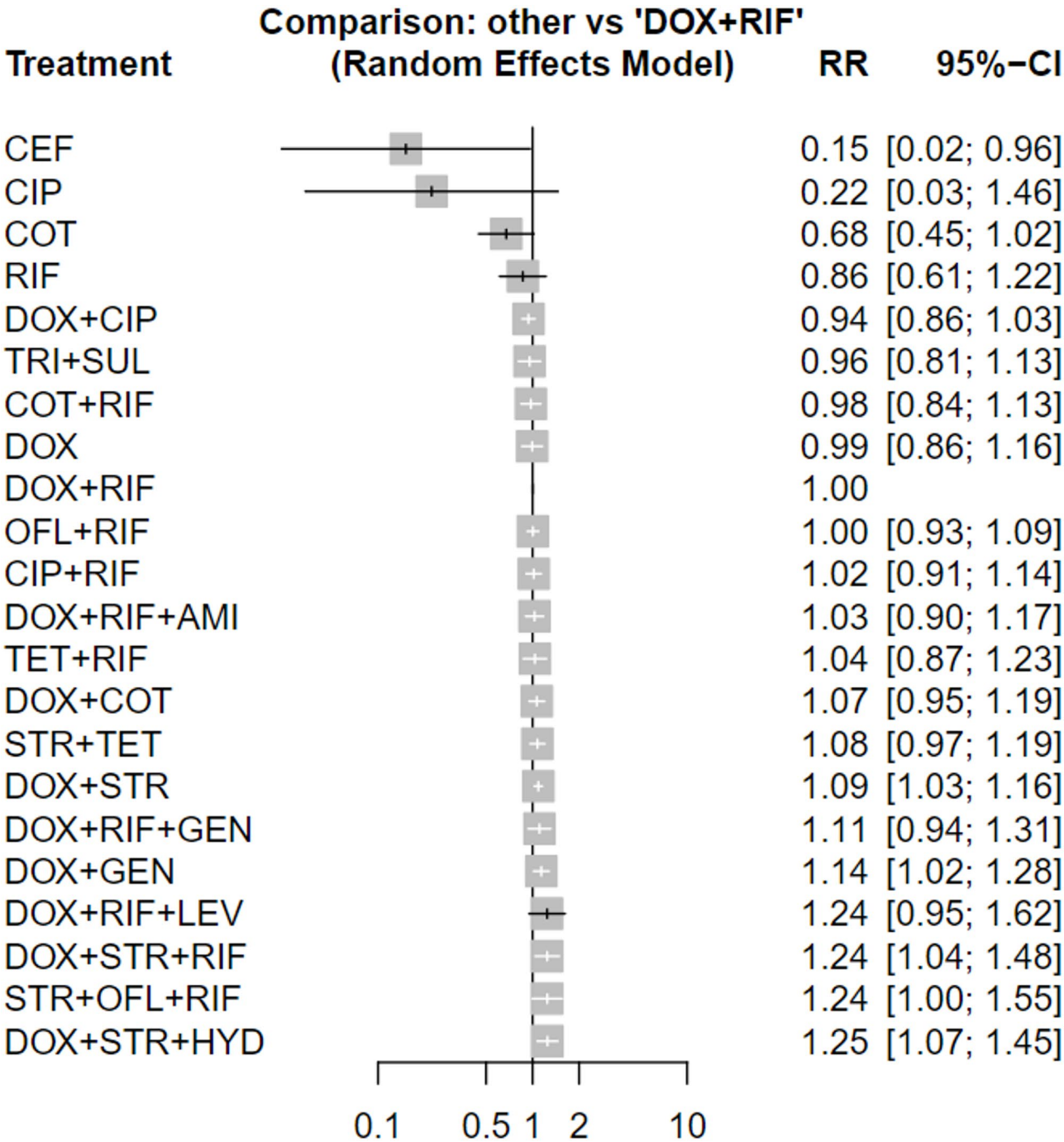


Fig. 4 Comparison of the RR effect size estimated from comparing the effectiveness of interventions with random effects model with Forest Plot from comparing the effectiveness of interventions

demonstrated the highest RR (1.25 [1.07, 1.45]), indicating a potential therapeutic advantage; however, further clinical interpretation is warranted.

Ranking of brucellosis drug regimens

We used the Sucra analysis in Stata to describe the ranking results. The SUCRA analysis (Figure S3) reveals that specific combination therapies, including DOX+STR+HYD (O), DOX+STR+RIF (P), STR+OFL+RIF (S), and DOX+RIF+LEV (M), achieve higher SUCRA values, indicating that they are among the most effective treatment options. In contrast, single-drug regimens, such as CEF (A), CIP (B), COT (D), and RIF (R), exhibit lower SUCRA values, suggesting diminished effectiveness compared to other therapeutic strategies. Positioned in the middle of the ranking in the SUCRA chart are regimens with moderate efficacy, such as DOX+RIF (J) and DOX+CIP (G). According to the analysis presented in the P-score (Figure S2) and forest plot (Fig. 4) results reveal that the treatment regimens DOX+STR+HYD, DOX+STR+RIF, and STR+OFL+RIF are ranked first, second, and third, respectively, in terms of therapeutic efficacy. These findings indicate that monotherapy regimens, including CEF, CIP, COT, and RIF, exhibit the lowest levels of therapeutic effectiveness in the management of brucellosis.

Consistency test

The results reveal a moderate level of heterogeneity, with $\tau^2 = 0.0032$ and $I^2 = 37.7\%$, indicating that 37.7% of the variability in treatment effects can be attributed to heterogeneity rather than chance, which is within the acceptable range [47]. The overall Q-test for heterogeneity within the study designs is significant ($p = 0.0357$), suggesting some variability among the studies. However, the Q-test for inconsistency between study designs is not significant ($p = 0.2923$), indicating that the consistency assumption of the network meta-analysis holds (Table S5). This suggests that the direct and indirect comparisons of treatment effects are consistent, thereby reinforcing the reliability of the network meta-analysis findings. These results underscore the robustness of the conclusions regarding the comparative effectiveness of different treatment regimens for human brucellosis. We employed the Net Heat Plot diagram to identify and select the appropriate model for our network analysis. The “random effects” model was chosen to analyze the data, and the results revealed an acceptable level of inconsistency within the network. Notably, none of the areas in the Net Heat Plot diagram displayed a dark red background, suggesting that the overall integrity of the model is significantly enhanced when the random effects model is applied (Fig. 5).

Consistency and effect size estimation for each design in the network were evaluated both directly and indirectly, utilizing forest plot diagrams and odds ratio (OR) effect sizes (Figure S4). To assess the validity of the results, publication bias was evaluated using funnel plot diagrams (Fig. 6 and Figure S5). The findings for both effect sizes are presented. Furthermore, the Egger test was employed to detect publication bias in the meta-analysis, based on funnel plot asymmetry. The relatively symmetrical distribution of data points around the center line, combined with an Egger test p -value of 0.6763, suggests the absence of significant publication bias in this meta-analysis. The symmetry of the funnel plot indicates that the included studies were distributed evenly, with minimal influence from publication bias. Furthermore, the nonsignificant Egger test p -value (0.6763) corroborates this finding, underscoring the robustness of the meta-analysis results (Fig. 6 and Figure S5).

Discussion

This network meta-analysis offers the first comprehensive evaluation of drug regimens for human brucellosis and reveals that triple-drug therapies—particularly DOX+STR+HYD—exhibit the highest treatment efficacy. The DOX+STR+HYD, STR+OFL+RIF, DOX+STR+RIF, and DOX+RIF+OFL regimens were ranked highest, while monotherapies such as CEF, CIP, COT, and RIF were least effective.

Clinical input suggests hydroxychloroquine may enhance recovery, potentially due to its anti-inflammatory properties and joint pain relief, but further research is warranted to confirm this hypothesis. Hydroxychloroquine may contribute to enhanced treatment response by alkalinizing phagolysosomes and improving antibiotic penetration into infected macrophages [48–51]. This mechanism aligns with previous findings in diseases like Q fever endocarditis, where the drug's combination with doxycycline resulted in improved outcomes [50]. Its anti-inflammatory properties may further alleviate symptoms such as arthralgia, which are common in brucellosis [49]. These effects have been noted anecdotally by the infectious disease experts within our research team.

The treatment of human brucellosis remains challenging despite several effective antibiotic regimens. Various therapeutic options, such as doxycycline, aminoglycosides, rifampicin, and quinolones, have been used in different combinations. Regimens like doxycycline with rifampicin or aminoglycosides have shown high success rates [11], while alternatives like quinolones or co-trimoxazole require further investigation [52]. Variability in treatment responses across patient populations highlights the importance of selecting effective therapies.

While CDC guidelines recommend dual-drug regimens such as doxycycline and rifampin [53], this study

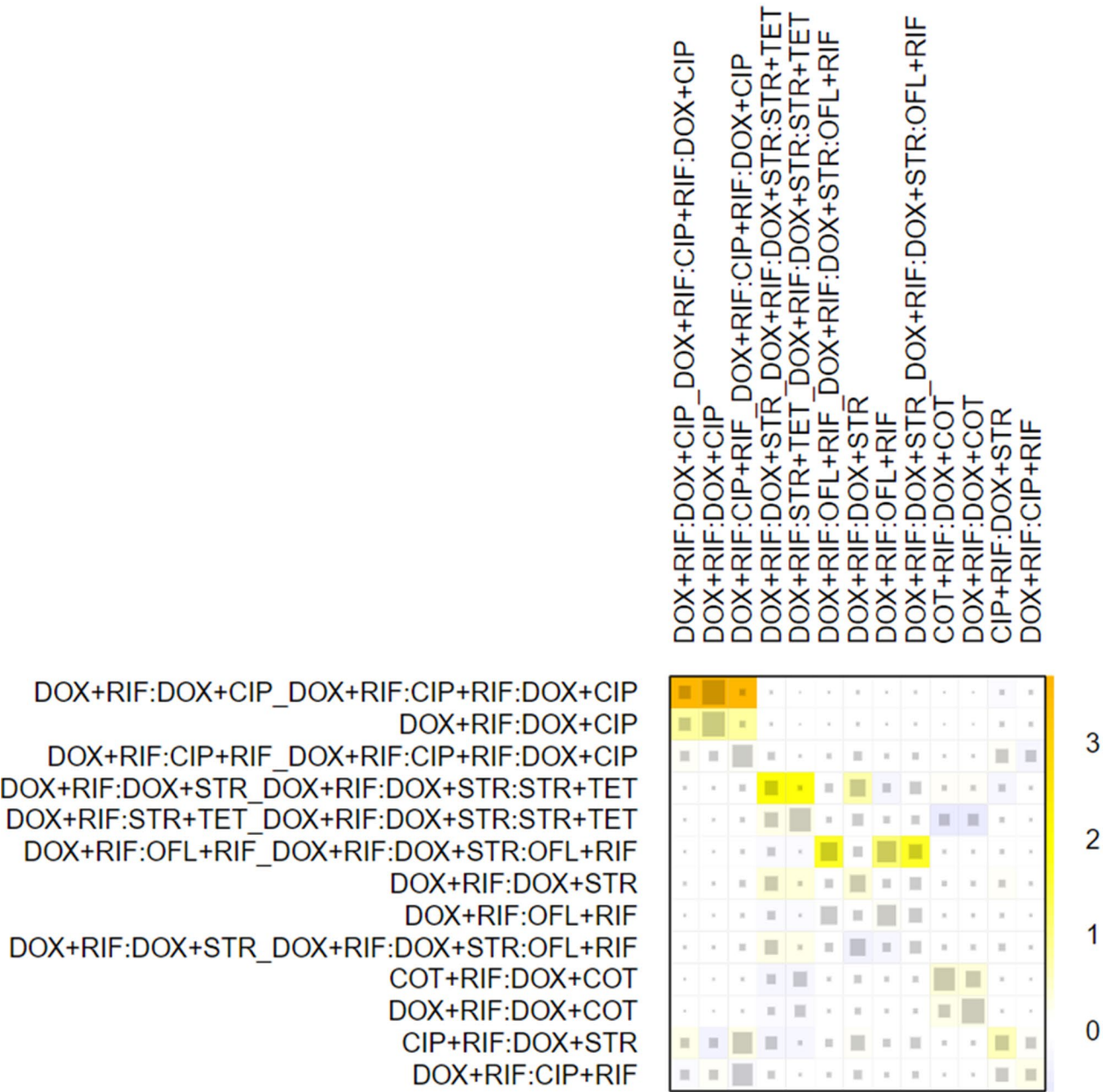


Fig. 5 Locating inconsistency in the network by Net Heat Plot diagram

supports the superior effectiveness of triple-drug regimens, particularly those including an injectable aminoglycoside. Our findings may prompt a reconsideration of international treatment guidelines. The addition of aminoglycosides enhances treatment efficacy by providing potent bactericidal activity, improved macrophage penetration, and inhibition of intracellular growth, leading to a greater reduction in bacterial load compared to rifampin and doxycycline [54].

The superiority of triple-drug regimens is supported by earlier pairwise and classical meta-analyses, which showed improved outcomes with 2–3 drug combinations

versus monotherapy [5, 7, 11, 12, 55, 56]. Studies by Bayindir Y et al. (2003) and Skalsky et al. (2008) also support the superior efficacy of triple-drug regimens in preventing relapse and achieving full recovery [11, 23]. Our findings are consistent with Iran’s national brucellosis treatment guidelines, which recommend combined regimens including two oral antibiotics and an injectable aminoglycoside as the preferred treatment option [9]. Recent systematic reviews have examined the effectiveness of these regimens, demonstrating their superiority compared to combinations of two drugs, and have proposed them as preferred treatment options [19, 23, 35].

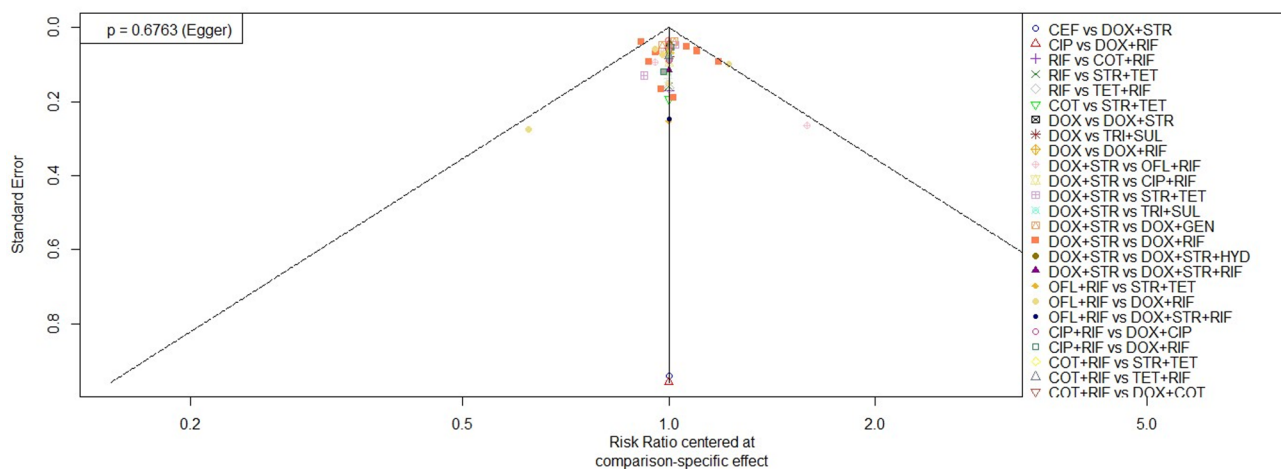


Fig. 6 Evaluation of the publication bias with Funnel Plot Diagram, by effect sizes RR

Nevertheless, practical challenges must be considered. Triple-drug regimens require more resources, and the injectable component may limit their use in low-resource settings. Dual-drug oral combinations, though easier to administer and more cost-effective, were less effective in our analysis. Policymakers should weigh these trade-offs carefully when updating national treatment guidelines to ensure both efficacy and feasibility.

The limitations of this study include moderate heterogeneity ($\tau^2 = 0.0032$, $I^2 = 37.7\%$) and variability in study quality. While the random-effects model and non-significant inconsistency tests ($p=0.2923$) support internal validity, potential biases in randomization, blinding, and reporting in some studies cannot be ruled out. Limited RCTs on regimens such as DOX+STR+HYD may restrict generalizability. However, the absence of publication bias ($p=0.6763$) and the network's consistency enhance the credibility of our findings [54].

Based on the findings of this network meta-analysis, the implementation of triple-drug regimens especially those containing an injectable aminoglycoside such as streptomycin should be strongly considered in regions with high brucellosis burden. These regimens have demonstrated superior efficacy in enhancing treatment outcomes. While current CDC and WHO guidelines [53, 57] prioritize dual-drug regimens for practicality and cost-effectiveness, healthcare systems, particularly in endemic areas, may benefit from revising their treatment protocols to incorporate these more effective combinations where feasible. Clinicians should assess the potential for adopting triple therapy, weighing it against local resource availability and patient adherence potential. Beyond clinical settings, public health authorities can leverage these findings to develop region-specific guidelines that better reflect the most effective treatment options revealed by this study. Implementation could include updating national treatment protocols, optimizing drug

procurement strategies to include aminoglycosides, and training healthcare professionals in the administration and monitoring of these regimens. These actions can contribute to reducing disease prevalence and recurrence, especially in rural or livestock-dependent communities where brucellosis remains endemic.

Conclusion

This NMA confirms the superior efficacy of triple-drug regimens, particularly those involving aminoglycosides like streptomycin, in treating human brucellosis. Based on our findings, the DOX+STR+HYD regimen may be considered an effective treatment option by physicians due to the presence of the hydroxychloroquine component, a result to be confirmed in future studies. While current CDC and WHO recommendations favor dual-drug regimens for practical reasons [53, 57], our findings suggest that, where feasible, implementation of triple-drug therapies could improve patient outcomes in high-burden areas. Public health authorities should consider updating treatment protocols and expanding access to aminoglycoside-based regimens. Further high-quality RCTs are essential to validate these findings and inform policy decisions.

Supplementary Information

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Supplementary Material 1.

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Clinical trial

Not applicable.

Authors' contributions

A.P. and M.H. did the study design and study framework. A.P. and M.H. and S.M. collected primary data. A.P. and M.H. conducted a risk assessment of the studies. A.P. performed data analysis and interpretation. A.P. and M.H. and S.M. and R.N. Manuscript writing. All authors reviewed the manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors provided written informed consent to publish. All images in this article have been approved by me and I have signed an informed consent form.

Competing interests

The authors declare no competing interests.

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