Drug resistance of clinical and environmental isolates of *Brucella* species in Iran: a meta-analysis

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Background: Brucellosis is a contagious and febrile disease endemic to Iran. Increased antibiotic resistance in endemic areas may lead to increased risk of treatment failure and the risk of disease relapse. This systematic review and meta-analysis was performed to determine the antibiotic susceptibility profiles of *Brucella* species isolated from clinical and environmental samples in Iran.

Methods: Using national and international databases and extracted keywords from the MeSH database, a fully computerized search was done until 11 June 2018. Of 385 collected studies on the prevalence of drug resistance of *Brucella* species isolated in Iran, six articles were included in the meta-analysis using predefined eligibility criteria.

Results: Overall resistance rates of *Brucella* species to different antibiotics in Iran were as follows: doxycycline: 0%, tigecycline: 5.1%, trimethoprim/sulfamethoxazole: 5.7%, ciprofloxacin: 2.7%, streptomycin: 5%, rifampin: 9.5%, tetracycline: 4.6%, gentamicin: 3.9%, moxifloxacin: 0%, erythromycin: 33.3%, azithromycin: 5.8% and ceftriaxone: 6.3%.

Conclusion: Our study revealed that the prevalence of drug resistance of *Brucella* species isolated from clinical and environmental samples in Iran was acceptable and low. However, care should be exercised in the use of common antibiotics for the treatment of brucellosis to prevent the spread of drug resistance.

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Keywords: antibiotic, *Brucella*, drug resistance, Iran, meta-analysis

Introduction

Brucellosis, also known as undulant fever or Malta fever, is a zoonotic disease that severely affects animal and human health. This disease is caused by a fastidious small Gram-negative coccobacilli bacteria from the genus *Brucella* [1]. Infection caused by this intracellular bacterial pathogen is contagious and variable in severity, and is transmitted to human from various hosts such as goats and sheep (*Brucella melitensis*), swine (*Brucella suis*) (severe disease), cattle (*Brucella abortus*) (mild disease) and dogs (*Brucella canis*) (mild disease) [1–3]. Transmission of the disease occurs in several ways including inhalation, consuming unpasteurized/raw dairy products and contact with infected animal tissues and is manifested in humans by nonspecific symptoms such as fever, chills, headache, fatigue, joint pain, low back pain, back pain, joint pain and body aches [1–3]. Human brucellosis can be divided into acute and chronic phases and affects in all age groups, thereby remaining as a public health issue especially in many developing countries in the Middle East, Mediterranean Basin, Southern Europe, North and East Africa, Southwest and Central Asia and Latin America. However, the disease has been eradicated in many developed

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countries due to extensive implementation of health and control programs [3,4]. Brucellosis is endemic in Iran in both humans and animals, especially in the West and Northwest regions of Iran [3]. According to the WHO estimates, more than half a million new cases of brucellosis occur every year across large parts of the globe [5]. Incidence of infection in Iran varies between 98 and 130 per 100 000 population [3]. Therefore, treatment of brucellosis is important in controlling the disease as well as in preventing relapse, miscarriage and some complications of brucellosis [6]. The gold standard antibiotic treatment recommended by WHO that was presented in 1986 is combination of oral doxycycline and rifampin for 6 weeks or intramuscular streptomycin for 2–3 weeks [7]. However, new treatment options such as quinolones (ciprofloxacin or ofloxacin), gentamicin, trimethoprim-sulfamethoxazole, tetracycline, macrolides and erythromycin are used to reduce the risk of drug resistance and treatment failure, reduce the high rate of recurrence (5–10%) after monotherapy and reduce serious side effects [7,8]. Similar the regimens are accepted as the preferred brucellosis treatment in Iran [8]. Several studies have reported the risk of increased antibiotic resistance in endemic areas that may lead to treatment failure and relapse [9].

The aim of the current systematic review and meta-analysis was to determine the drug resistance pattern of *Brucella* species isolated from human and animal samples in Iran.

Methods

For reporting of systematic reviews and meta-analyses, the Preferred Reporting Items for Systematic Review and Meta-Analyses checklist was used [10].

Search strategy

Comprehensive search in national and international databases including PubMed, Scopus, Google Scholar and ISI web of knowledge as well as SID (the Scientific Information Database) and Magiran was performed. The keywords used to identify English and non-English studies were 'drug resistance' OR 'antibiotic resistance' AND '*Brucella*' AND 'clinical sample' OR 'environmental sample' AND 'Iran'. The last date of search was 11 June 2018. Additional studies were identified by checking the reference lists of the retrieved articles and hand searching of journals.

Study selection

After a full electronic search, we established a library containing studies reporting drug resistance of clinical and environmental isolated *Brucella* species in Iran. Identified cross-sectional studies were selected based on the eligibility criteria as follows: publication in English or Persian languages, reporting drug resistance of *Brucella* species from clinical and environmental specimens and limited to Iran.

Data extraction

To obtain all relevant information and avoid data entry errors, data extractions were performed by two authors. Authors, year of the study, location of the study, sample type, specimen type, methods used for bacterial identification, number of isolated strains, *Brucella* species type, methods used for antimicrobial susceptibility testing and antibiotic resistance rate of bacteria to various used antibiotics were the main collected data from each of the included studies. In addition, quality of the selected cross-sectional studies was performed based on Newcastle-Ottawa scale (Table 1).

Statistical analysis

To analyze and interpret collected data from included studies, we used Comprehensive Meta-Analysis software version 2.2 (Biostat, Englewood, New Jersey, USA). Depending on the presence or absence of heterogeneity in the study results, fixed-effects or random-effects approach was applied to pool the data. The I^2 statistic was used as an index of heterogeneity. An I^2 value of less than 25% was considered as 'low heterogeneity' suggesting the use of fixed-effects model (Table 2). Antibiotic resistance rate of *Brucella* species collected from human and animal

Table 1. Quality of the included studies according to the Newcastle-Ottawa scale.

	Selection[a]				Comparability[b]	Outcome[c]	
Study	Representativeness of the sample	Sample size	Nonrespondents	Ascertainment of the exposure	Comparability of outcome groups	Assessment of the outcome	Statistical test
Irajian *et al.*	*	*	–	**	*	*	–
Irajian *et al.*	*	*	–	**	*	*	–
Asadi *et al.*	*	*	–	**	*	*	*
Farazi *et al.*	*	*	–	**	*	*	*
Rashidi *et al.*	*	*	–	**	*	*	–
Ashrafganjooy *et al.*	*	*	–	*	*	*	–
Razzaghi *et al.*	*	*	–	**	*	*	–

[a]Maximum 5 stars.
[b]Maximum 2 stars.
[c]Maximum 3 stars.

Table 2. Antibiotic susceptibility profile of *Brucella* species in both human and animal samples in different provinces of Iran.

	Antibiotic resistance (%) (95% CI)											
Province	DOX	TIG	TMP-SXT	CIP	STR	RIF	T	GM	MXF	E	AZ	CRO
Tehran	0	10.5 (4–24.9)	3 (0.7–11.1)	0	0	0	6 (0.6–39.3)	6.5 (1.8–20.4)	ND	ND	ND	ND
Hamadan	0	ND	0	0	0	0	ND	0	0	ND	ND	ND
Markazi	0	0	10 (3.3–26.8)	6.7 (1.7–23.1)	6.7 (1.7–23.1)	33.3 (19–51.6)	0	0	ND	33.3 (19–51.6)	16.7 (7.1–34.3)	20 (9.3–37.9)
Kurdistan	0	ND	ND	ND	11.1 (2.8–35.2)	83.3 (59.1–94.5)	0	ND	ND	ND	ND	ND
Kerman	ND	ND	55.6 (25.1–82.3)	ND	22.2 (5.6–57.9)	22.2 (5.6–57.9)	ND	ND	ND	ND	ND	ND
Kashan	0	ND	0	0	0	0	ND	ND	ND	ND	0	0
Total	0	5.1 (1.3–18.2)	5.7 (1.2–22.7)	2.7 (1–7.1)	5 (1.9–12.5)	9.5 (1.9–36.9)	4.6 (1.2–16.4)	3.9 (1.2–11.6)	0	33.3 (19–51.6)	5.8 (0.4–51.3)	6.3 (0.3–59.3)
Heterogeneity I^2 (%)	0	33.2	77.5	0	40.4	85.4	39	28.8	0	0	74.2	78

AZ, azithromycin; CIP, ciprofloxacin; CRO, ceftriaxone; DOX, doxycycline; E, erythromycin; GM, gentamicin; MXF, moxifloxacin; ND, not determined; RIF, Rifampin; STR, streptomycin; T, tetracycline; TIG, tigecycline; TMP-SXT, trimethoprim/sulfamethoxazole.

samples were expressed as percentage and 95% confidence intervals (95% CIs) in different cities. Finally, evaluating publication bias was done using funnel plots (Fig. 1).

Results

Figure 2 describes the process for selecting studies using eligibility criteria. Briefly, a total of 385 studies on the prevalence of drug resistance of clinical and environmental isolates of *Brucella* species were collected from national and international databases. After reviewing the titles, abstracts and full texts of articles and removing congress abstracts, reviews, duplicate publications and articles with insufficient information, 380 articles were excluded from the meta-analysis. In addition, one study was included by checking the reference lists of articles and the meta-analysis was done with 6 included studies (Fig. 2). Included studies were conducted in Tehran (two studies), Hamadan (one study), Markazi (one study), Kurdistan (one study), Kerman (one study) and Kashan (one study).

As shown in Table 3, disk diffusion, microbroth dilution, *E* test and agar dilution were the most widely used methods for testing the antimicrobial susceptibility of *Brucella* species. *B. melitensis* was the most common isolated *Brucella* species in both human and animal samples.

As shown in Table 2, the prevalence of antibiotic resistance of *Brucella* species in both human and animal

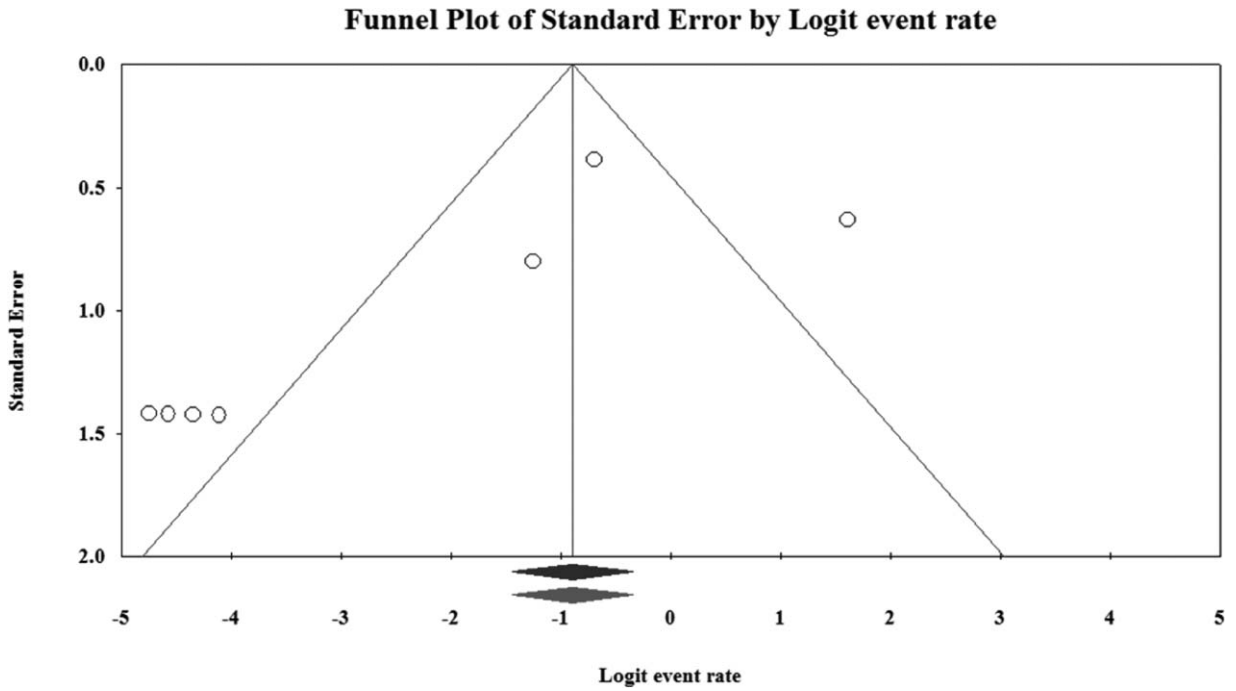

Fig. 1. Funnel plot of the meta-analysis on the prevalence of antibiotic resistance of *Brucella* species to rifampin in Iran.

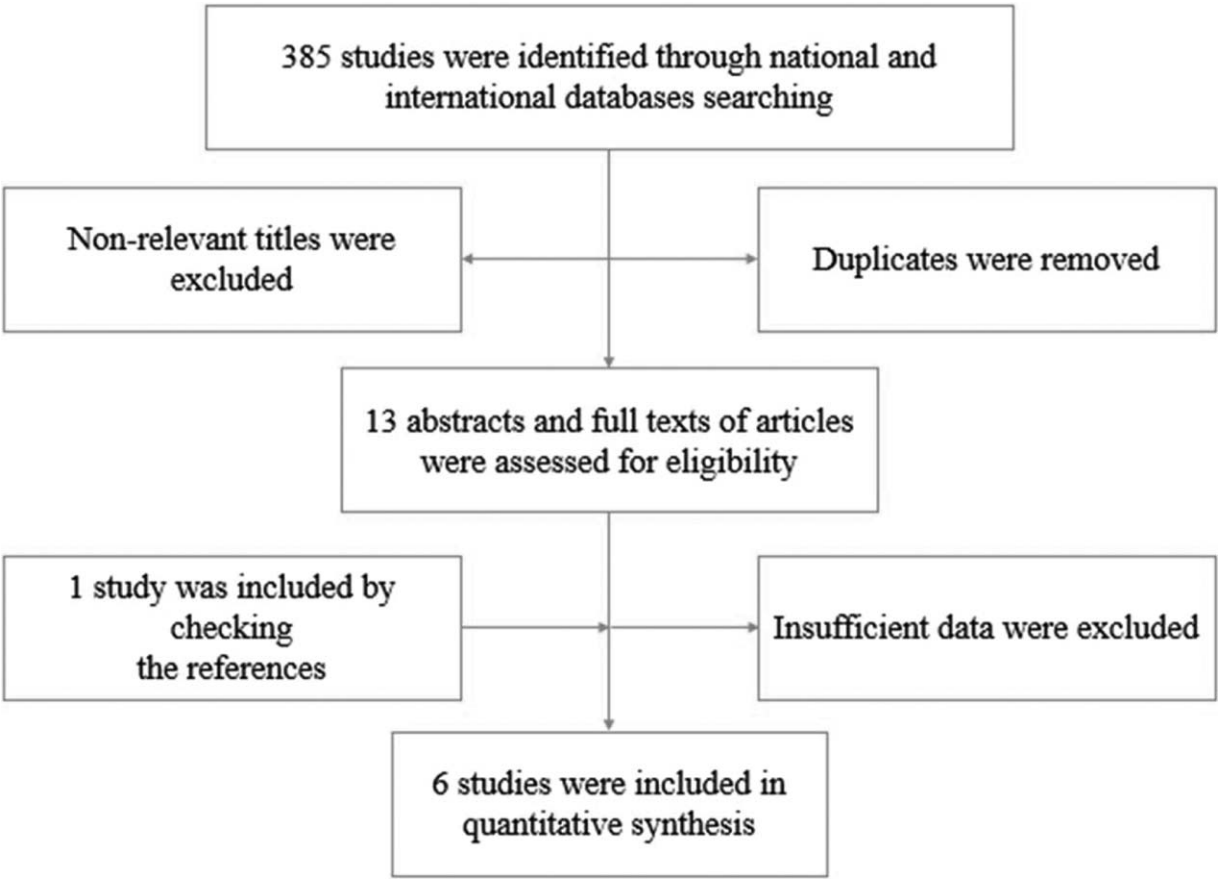

Fig. 2. Study flow diagram.

samples in Iran was as follows: doxycycline (0%), tigecycline (5.1%; 95% CI: 1.3–18.2), trimethoprim/sulfamethoxazole (5.7%; 95% CI: 1.2–22.7), ciprofloxacin (2.7%; 95% CI: 1–7.1), streptomycin (5%; 95% CI: 1.9–12.5), rifampin (9.5%; 95% CI: 1.9–36.9), tetracycline (4.6%; 95% CI: 1.2–16.4), gentamicin (3.9%; 95% CI: 1.2–11.6), moxifloxacin (0%), erythromycin (33.3%; 95% CI: 19–51.6), azithromycin (5.8%; 95% CI: 0.4–51.3) and ceftriaxone (6.3%; 95% CI: 0.3–59.3).

Discussion

Brucellosis infection is still endemic in Iran and has imposed economic and public health costs to both healthcare system and livestock industry [3,8]. In Iran, similar to other parts of the world, *B. melitensis* is the major cause of human brucellosis [16]. Therefore, timely treatment with single or combined regimens of antibiotics is pivotal to ensure optimum effectiveness of the treatment [17].

The major therapeutic regimens used for brucellosis infection include monotherapy and dual or triple drug therapy with doxycycline, rifampin, streptomycin and gentamicin as first-line treatments [16,18]. Several studies have assessed monotherapy and showed some efficacy and high relapse rates [6,16]. Nevertheless, in patients with a low risk of relapse, monotherapy is a cost-effective alternative regimen [6]. Combination therapy is the most effective regimen and recommended in many studies [6,7,16]. In the current study, we found that 100% of isolated *Brucella* species in Iran were susceptible to doxycycline (Table 2). Similar findings were noted in Egypt, Brazil, Malaysia, Mexico and Peru [9,17,19–21]. Doxycycline is the drug of choice included in various combination regimens with streptomycin, rifampicin and gentamicin [7,16]. According to WHO (1986) and Ioannina (2007) recommendations, doxycycline (6 weeks) and rifampicin (6 weeks) or streptomycin (2–3 weeks) are as first-line regimens for the treatment of uncomplicated brucellosis [16,18]. Total resistance rate of *Brucella* species to rifampicin was low in Iran (9.5%) (Fig. 3). Studies from Brazil, Mexico and Peru investigated antibiotic resistance profile and obtained similar results [17,20,21]. Considering the potential risk of inducing resistance to rifampicin in some endemic regions of tuberculosis (TB), using this antibiotic is challenging especially in Iran where TB and brucellosis are endemic [22,23]. Therefore, in Kurdistan (83.3%), Markazi (33.3%) and Kerman (22.2%) provinces of Iran

Table 3. Characteristics of studies included in this meta-analysis.

First author (Ref)	Year	Area	Sample type	Specimen type	Bacterial identification method(s)	Strains, *n*	Isolated strains	AST	Antibiotic resistance, *n*											
									DOX	TIG	TMP-SXT	CIP	STR	RIF	T	GM	MXF	E	AZ	CRO
Irajian *et al.* [8]	2010–2015	Tehran	Human	Blood CSF	Microbiological methods[a]	30	*B. melitensis* *B. abortus*	*E* test Microbroth dilution	0	0	1	0	0	0	4	3	ND	ND	ND	ND
Irajian *et al.* [8]	2010–2015	Tehran	Animal	Blood Liver Spleen	Microbiological methods	38	*B. melitensis* *B. abortus* *B. suis*	*E* test Microbroth dilution	0	4	1	0	0	0	0	1	ND	ND	ND	ND
Asadi *et al.* [11]	2013–2014	Hamadan	Human	Blood Synovial fluid Bone marrow	Microbiological methods	57	*B. melitensis*	*E* test	0	ND	0	0	0	0	ND	0	0	ND	ND	ND
Farazi *et al.* [12]	2014	Markazi	Human	Blood	Microbiological methods	30	*B. melitensis*	Disk diffusion	0	0	3	2	2	10	0	0	ND	10	5	6
Rashidi *et al.* [13]	2011	Kurdistan	Human	Blood	Microbiological methods	18	*B. melitensis*	Disk diffusion Agar dilution	0	ND	ND	ND	2	15	0	ND	ND	ND	ND	ND
Ashrafganjooy *et al.* [14]	2015	Kerman	Animal	Raw milk	Microbiological methods	9	*B. melitensis*	*E* test Agar dilution	ND	ND	5	ND	2	2	ND	ND	ND	ND	ND	ND
Razzaghi *et al.* [15]	2011–2013	Kashan	Human	Blood Synovial fluid	Microbiological methods	48	*B. melitensis*	*E* test	0	ND	0	0	0	0	ND	ND	ND	ND	0	0

AST, antimicrobial susceptibility testing; AZ, azithromycin; CIP, ciprofloxacin; CRO, ceftriaxone; CSF, cerebrospinal fluid; DOX, doxycycline; E, erythromycin; GM, gentamicin; MXF, moxifloxacin; ND, not determined; RIF, rifampin; STR, streptomycin; T, tetracycline; TIG, tigecycline; TMP-SXT, trimethoprim/sulfamethoxazole.

[a]Blood culture medium (BACTEC automated blood culture system and *Brucella* agar/broth culture) for 7–30 days along with Gram staining, oxidase, catalase, urease and growth characteristics as well as *Brucella* antibodies detection with the Wright, Coombs Wright and 2-mercaptoethanol agglutination tests.

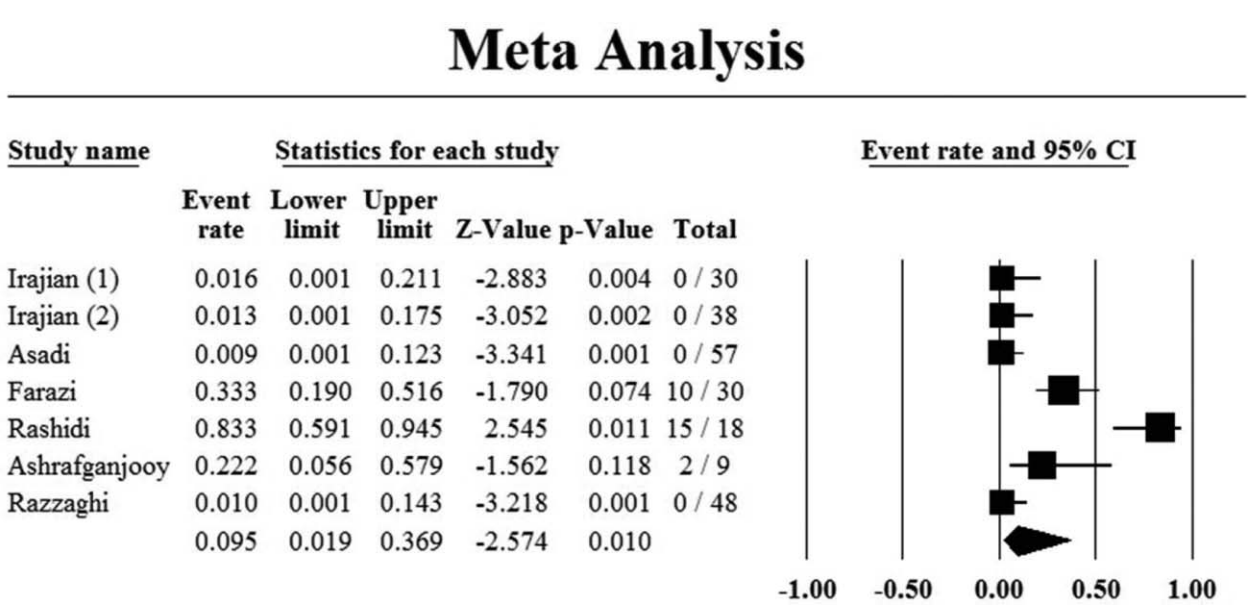

Fig. 3. Forest plot of the meta-analysis on the prevalence of antibiotic resistance of *Brucella* species to rifampin in Iran.

where there is a high resistance rate to rifampicin, using this antibiotic to treat infections is not recommended. Another valuable anti-TB agent is streptomycin. Similar to rifampicin susceptibility profile, streptomycin resistance rate in Iran was low (5%). Similar have been reported in Egypt, Brazil, Malaysia and Mexico [9,17,19,20]. However, treatment of brucellosis with streptomycin has some serious side effects including hearing disorders, nephrotoxicity and toxic effects on the nervous system and can be limited due to parenteral administration and streptomycin shortage [8,16]. An alternative regimen that has been recommended by WHO for the treatment of brucellosis is tetracycline (6 weeks) and streptomycin (2–3 weeks) [18]. In the current study, resistance rate of *Brucella* species to tetracyclines including tetracycline (4.6%) and tigecycline (5.1%) was low. Resistance rates were similar to those reported in Egypt, Malaysia and Mexico [9,19,20].

Second-line regimen recommended by Ioannina for the treatment of brucellosis is combination of doxycycline (6 weeks) and gentamicin (1 week) [18]. Our study revealed that 3.9% of *Brucella* species were resistant to gentamicin in Iran. Gentamicin resistance rate in Iran was similar to those reported from Brazil (3.4%) and Malaysia (0%) [17,19]. Therefore, efficacy of these aminoglycoside drugs, streptomycin and gentamicin, is high against brucellosis in Iran. Despite the low resistance to both streptomycin and gentamicin antibiotics in Iran, the use of gentamicin is preferred for two reasons: first, the wider availability of this drug and second, to prevent increasing streptomycin resistance in Iran, an endemic country for TB [16].

In addition, the optimal treatment regimens recommended by WHO and Ioannina are cotrimoxazole, ofloxacin and ciprofloxacin [18]. Cotrimoxazole monotherapy has been proposed by some sources for children under the age of 8 years and for pregnant women with contraindications for tetracyclines and quinolones, while WHO recommends rifampicin monotherapy [18,24]. In Iran, resistance rate of *Brucella* species to cotrimoxazole (5.7%) and ciprofloxacin (2.7%) was low. Therefore, cotrimoxazole is an appropriate and low-cost alternative in Iran.

New antimicrobial agents such as macrolides are also used in Iran to decrease the toxic side effects, relapses and drug resistance associated with commonly used antibrucellosis drugs [8]. However, resistance rate to erythromycin was high in Iran (33.3%).

Conclusion

The efficacy of antibiotic regimens for the treatment of brucellosis is different among various regions of the world. However, it seems that WHO-recommended regimens are still efficient in Iran. Based on the results of this meta-analysis, except erythromycin, resistance rate of *Brucella* species to commonly used antibrucellosis drugs in Iran was acceptable and low. We recommend that identification of the main mechanisms responsible for the induction of resistance in *Brucella* species would be helpful guide the choice of antibiotics in Iran and other parts of the world.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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