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REVIEW

The perspective of antibiotic therapeutic challenges of brucellosis in the Middle East and North African countries: Current situation and therapeutic management

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Abstract

Brucellosis is among the most prevalent zoonotic infections in Middle Eastern and North African (MENA) countries, critically impacting human and animal health. A comprehensive review of studies on antibiotic susceptibility and therapeutic regimes for brucellosis in ruminants and humans in the MENA region was conducted to evaluate the current therapeutic management in this region. Different scientific databases were searched for peer-reviewed original English articles published from January 1989 to February 2021. Reports from research organizations and health authorities have been taken into consideration. *Brucella melitensis* and *Brucella abortus* have been reported from the majority of MENA countries, suggesting a massive prevalence particularly of *B. melitensis* across these countries. Several sporadic cases of brucellosis relapse, therapeutic failure, and antibiotic resistance of animal and human isolates have been reported from the MENA region. However, several studies proved that brucellae are still in-vitro susceptible to the majority of antibiotic compounds and combinations in current recommended World Health Organization (WHO) treatment regimens, for example, levofloxacin, tetracyclines, doxycycline, streptomycin, ciprofloxacin, chloramphenicol, gentamicin, tigecycline, and trimethoprim/sulfamethoxazole. The current review presents an overview on resistance development of brucellae and highlights the current knowledge on effective antibiotics regimens for treating human brucellosis.

KEYWORDS
antibiotic resistance, brucellosis, MENA, therapeutics

1 | BACKGROUND

Brucellosis is a global zoonotic disease characterized by a severe multitude of non-specific and multi-systematic infections in humans and animals, resulting in public health concerns and substantial economic losses in livestock (X. H. Wang & Jiang, 2020). It has been estimated that >500,000 new cases of human brucellosis are identified annually (O'Callaghan, 2020). However, this number is incorrect because

it excluded Chinese cases, and reports of many countries lack accurate case records, or the simple infrastructure to diagnose fever of unknown origin (FUO) is missing (O'Callaghan, 2020). The majority of the cases have been reported from the Middle East, Central Asia, Mediterranean countries, India, Central and South America, and Mexico (Bagheri Nejad et al., 2020; Musallam et al., 2016; Pourbagher et al., 2006). Brucellosis is caused by facultative intracellular, non-motile, Gram-negative bacteria belonging to order *Rhizobiales*, class *Alphaproteobacteria*, family *Brucellaceae*, and genus *Brucella* (*B.*) (Corbel, 1997). Of the 12 known *Brucella* species, only *B. abortus*, *B. melitensis*, *B. suis* biovars 1 and 3, and to some extent, *B.* canis are human pathogens. Moreover, recently, a new species has been isolated from a blood sample of a man and was named *B. pseudogrignonensis* (Li et al., 2021). Additionally, the environmental genus *Ochrobactrum* is also closely related to the genus *Brucella*. However, the exact positioning of *Brucella* among *Ochrobactrum* species is not conclusively resolved yet (Leclercq et al., 2019; Ryan & Pembroke, 2020). Brucellosis in livestock has significant socio-economic effects in low- and middle-income communities, especially in the Middle East (Franc et al., 2018; Hotez et al., 2012; Musallam et al., 2016; Rossetti et al., 2017). The disease is mainly associated with infertility, fetal death, late-gestation abortion, and decreased productivity in livestock (El-Diasty et al., 2021; Poester et al., 2013). Brucellosis is characterized by high morbidity rates in humans and animals and presents a major public health hazard in many developing countries (Dadar et al., 2018). Humans acquire the infection either through direct contact with diseased animals or by consuming infected raw dairy products (Dadar et al., 2018). Acute human brucellosis is challenging for clinicians and scientists due to missing of biomarkers for correlating with disease severity, treatment response, progression, improvement of therapeutic regimens, and understanding the mechanisms of pathogenicity of brucellae (Franco, Mulder, Gilman, et al., 2007). The symptoms of human acute brucellosis are unspecific, that is, fatigue, sweating, undulating fever, chills, myalgia, and arthralgia (Franco, Mulder, Gilman, et al., 2007; Megid et al., 2010). The disease has the tendency to shift towards persistence and chronicity if it is not diagnosed early and treated correctly during the acute phase (Doganay & Aygen, 2003). In humans, *B. melitensis* is the most commonly isolated agent, followed by *B. suis*, whereas *B. abortus* is associated with the mildest syndrome (Galinska & Zagórski, 2013).

Treatment of animal brucellosis is not applicable as all positively tested animals should be slaughtered. However, meat of slaughtered animals is still consumed in several regions of the Middle East after removing reproductive organs and draining lymph nodes. It has been estimated that around 3.5 billion people have a permanent risk of acquiring brucellosis, resulting in significant public health impacts and economic losses due to treatment costs and reduced work efficiency (Bosilkovski et al., 2021; Rossetti et al., 2017). Furthermore, the absence of adequate vaccines hinders eradication of the disease in some countries where test and slaughter practices are not applicable. Human vaccines are not on the market as well. Thus, treatment is essential for managing brucellosis in humans (Bosilkovski et al., 2021). Brucellosis therapy aims to stop symptoms, decrease sequalae, and avoid relapses (Al Dahouk & Nöckler, 2011; Doganay & Aygen, 2003). Well-timed, early, and effective antimicrobial therapy for brucellosis guarantees successful clearance. Recently, several reports of relapses of *Brucella* infections after well-timed treatment have been published. It remains unclear whether these relapses were caused by the development of acquired resistance or sequestration within infected sites such as parenchymatous organs and bone. This finding is alarming as World Health Organization (WHO) recommended regiemes obviously failed, and tens of thousands of new human cases are to be expected annually posing remarkable political and socio-economic impacts on MENA countries. Thus, reviewing the published data on therapeutic regiemes in use and evaluating the resistance profiles of brucellae in MENA countries will generate helpful information regarding need for future amendment of treatment protocols of human brucellosis.

2 | METHODOLOGY

2.1 | Study region

The countries of the Middle Eastern region comprise about 5.2% and 5.3% of the land area and population in the world, respectively (Bagheri Nejad et al., 2020; Pappas & Memish, 2007). The Middle East includes 17 countries, namely Egypt, Oman, Kuwait, Saudi Arabia, Bahrain, Iran, Israel, Lebanon, Jordan, Palestine, Qatar, Iraq, Syria, Yemen, United Arab Emirates, Cyprus, and Turkey. Seven countries are located in the UN subregion of North Africa, including Egypt, Algeria, Morocco, Libya, Tunisia, Western Sahara, and Sudan. Thus, geographically, this region covers most of the Arab World of North Africa and West Asia.

The English acronym of MENA refers to the Middle East and North African countries. Nineteen countries are generally considered part of the MENA region, including Egypt, Iran, Algeria, Israel, Qatar, Bahrain, United Arab Emirates, Kuwait, Iraq, Morocco, Lebanon, Syria, Jordan, Palestine, Libya, Oman, Saudi Arabia, Yemen, and Tunisia. In addition, some countries such as Cyprus, Ethiopia, Somalia, Sudan, and Turkey are included in the MENA group depending on usage. Numerous similarities in livestock management systems, culture, and environmental conditions are present among these MENA countries. Approximately 6% of the goats, sheep, and cattle of the world are found in the Middle Eastern region in which the main livestock are small ruminants with around 85% of the total reported livestock (Maxwell & Bill, 2008). This highlights the critical aspects of small ruminants, the primary source of *B. melitensis* in animal farming and food supply in the Middle Eastern region. The density of sheep and goats in these countries is almost twice that of those reported in the rest of the world.

2.2 | Data acquisition and source

This comprehensive systematic review was performed using public scientific databases, including Cochrane, Scopus, and PubMed, to retrieve articles reporting antibiotic susceptibility studies on brucellae and therapeutic challenges of brucellosis treatment in animal and human populations from January 1989 to February 2021, in different

populations and countries of the MENA region. The most recent data on documented brucellosis cases in animal and human populations were also retrieved from official online databases of the World Organization for Animal Health (OIE) and Food and Agriculture Organization of the United Nations (FAO) in the MENA region.

2.3 | Search strategy and data extraction

All national and international studies discussing antimicrobial sensitivity testing of *Brucella* isolates published in English and the native language were collected. The scientific sites, for example, research gate, google scholar, have been searched for any research, studies, or experiments on the sensitivity of antibiotics to brucellae. In countries lacking published scientific data such as Libya, Tunisia, Kuwait, Chad, Niger, researchers working on brucellae, local research organizations, or health authorities were contacted through emails and telephone calls to confirm that no studies were done or known. Data on diagnosis of brucellosis including serological tests, molecular techniques, and bacterial isolation on specific media were also requested. Full research articles, case reports, review articles, conference abstracts, letters, commentaries, and editorials carried out on human and animal isolates in any country of the MENA region were included.

The title and abstract of each publication were checked, and all studies discussing therapeutic regimes in human cases, treatment trials in animal cases, in-vivo antibiotic sensitivity testing for isolates from animal and human origins, molecular detection of resistance genes/mutations, or in-silico detection of AMR using the whole-genome of brucellae were included, if selected, the full text was carefully analyzed. The following keywords were used to perform the literature search: brucellosis, sensitivity testing, treatment, antibiotic susceptibility, and antimicrobial activity . The following information was extracted from the full text of each study: country of origin, year of sampling or isolation, first author and year of publication or report, study population and source of samples (humans, animal hosts, foodstuff), number of isolates and type of *Brucella* spp., and resistance profiles of the recovered strains including phenotypic testing (names and percentages of susceptible and resistance antibiotics found, method used in antibiotic testing). If any of the articles provided results of whole-genome, the detection of resistance genes or the presence of mutations in the genomes was checked.

3 | RESULTS

3.1 | Data analysis

A total of 51 published studies from 10 countries (Egypt, Iran, Algeria, Lebanon, Iraq, United Arab Emirates, Qatar, Syria, Turkey, and Saudi Arabia) discussing susceptibility testing of *Brucella* isolates were used for the analysis. Of them, 38 studies investigated isolates from humans, 11 studies of animal origins and two studies from Egypt (Wareth et al., 2021) and Iran (Irajian et al., 2016) included isolates from humans and animals. The studies have been carried out between 1989 and 2021. Most of the studies have been carried out on isolates from Turkey (n = 21), followed by Iran (n = 10) and Saudi Arabia (n = 6). Nine studies have been carried out on isolates from Egypt (n = 3), Iraq (n = 3), and Syria (n = 3). In addition, one study has been carried out on isolates from Algeria, Qatar, Lebanon, and United Emirates, and another study has been carried out in Norway on isolates from the Middle East, including Iraq, Turkey, Israel, Somalia, and Ethiopia (Johansen et al., 2018). The comparative data on antibiotic resistance of *Brucella* isolates from humans and animals are shown in Tables 1 and 2, respectively.

Time of sampling was mentioned in 38 studies only. Most of the studies were carried out on *B. melitensis* isolates (n = 47), while *B. abortus* isolates were included in 13 studies, *Brucella ovis* in one study, and two studies did not differentiate the species. The E-test was most often used (n = 34), followed by the disk diffusion test (n = 11), and a few studies have used the broth microdilution test (n = 8). Resistance to rifampicin was reported in isolates from humans in 22 studies and isolates from animals in eight studies. Resistance to streptomycin was reported in human isolates of four studies and animal isolates of seven studies. Resistance to trimethoprim/sulfamethoxazole (T/S) was described on isolates from humans in six studies and on animal isolates in three studies. Resistance to azithromycin (AZI) was seen in isolates from humans in four studies but only in one study on isolates from animals. Resistance to ceftriaxone (CEF) was reported on human isolates of four studies and animal isolates of three studies (Table 3). No data were obtained from research or public health authorities in Bahrain, Kuwait, Morocco, Jordan, Palestine, Libya, Oman, Yemen, Tunisia, Cyprus, and Sudan.

3.2 | Brucellosis and *Brucella* control in the MENA region

Animal and human brucellosis are endemic in most countries of the Middle Eastern region, such as Iran, Saudi Arabia, Iraq, Egypt, and Turkey, and Syria is reported to have the world's highest incidence rate (Musallam et al., 2016; Pappas & Memish, 2007). The occurrence of *B. melitensis* and *B. abortus* has been well documented in the majority of Middle Eastern countries (Musallam et al., 2016). The seroprevalences in small ruminant populations are the highest worldwide. Human cases have variable annual incidence from 0.5 to 88.6 rate per 100,000 inhabitants, and brucellosis is likely associated with the consumption of unpasteurized dairy products or occupationally exposure to infected ruminants (Musallam et al., 2016). However, the actual prevalence of brucellosis in animals and humans may be far higher than the published data because of the uncompleted data associated with misdiagnosis and reporting errors (Benkirane, 2006; Hotez et al., 2012; Musallam et al., 2016; Pappas & Memish, 2007). There are few valid data on the actual prevalence of brucellosis in ruminant livestock of the Middle Eastern countries available (Benkirane, 2006). Different studies showed that older animals and mixed farming systems of small ruminants were significantly

TABLE 1 Comparative data of studies on antibiotic therapeutic regimes using *Brucella* spp. of human origin in the Middle Eastern and North African (MENA) countries ($n = 40$)

Country	*Brucella* spp. (No.)	Susceptible strains (% or No.)	Non-susceptible strains (% or No.)	Method	References
Middle East	*B. melitensis* (23)	DOX, STR, T/S, GEN	RIF (23)	E-test, broth microdilution	(Johansen et al., 2018)
Egypt	*B. melitensis* (10) *B. abortus* (2) 2018–2020	CMP, CIP, DOX, GEN, LEV, STR, TET, T/S, TGC (100%)	AZI (8), RIF (10)	Microdilution, Disk diffusion	(Wareth et al., 2021)
Egypt	*B. melitensis* (355) 1999–2007	TET, DOX, T/S, STR, CIP (100%)	RIF (277) 64% and CEF (7) 2%	E-test	(Abdel-Maksoud et al., 2012)
Iraq	*B. melitensis* *B. abortus*	STR (100%), TET (100%), GEN (100%), KANN (91.6%), TOB (91.6%), CMP (82.3%), ERY (50%)	PEN (100%)	Disk diffusion	(Abed Mohamad, 1998)
Iran	*B. melitensis* (11) 2017–2019	RIF	No	E-test	(Bazrgari et al., 2020)
Iran	*B. melitensis* (60) 2016–2018	CEF, DOX, STR, T/S, GEN (100%)	RIF (1) and A/S (11)	E-test Disk diffusion	(Alamian et al., 2019)
Iran	*B. melitensis* (30) 2014	TET, GEN, TGC (100), DOX (93.3%), RIF (44.7%), STR (86.7%), CIP (80%), COT (76.7%), CEF (73.3%)	T/S (3), CIP (2), STR (2), RIF (10), ERY (10), AZI (5), CEF (6)	E-test, Disk diffusion	(Farazi et al., 2018)
Iran	*Brucella* spp. (6/385 studies) meta-analysis until 2018	DOX (100%), CIP (97.3%), GEN (96.1%), STR (95%), TET (95.4%),	TGC (5.1%), T/S (5.7%), CIP (2.7%), STR (5%), RIF (9.5%), TET (4.6%), ERY (33.3%), AZI (5.8%), CEF (6.3%).		(Khademi et al., 2018)
Iran	*B. melitensis* (48) 2011–2013	DOX, CIP, T/S, STR, AZI, CEF (100%)	RIF (1)	E-test	(Razzaghi et al., 2016)
Iran	*B. melitensis* (149) 2013–2014	DOX, STR, GEN, CIP, MOX (100%)	RIF (35%), T/S (3.5%)	E-test	(Hashemi et al., 2016)
Iran	*B. melitensis* (57) 2013–2014	DOX, CIP, MOX, STR, GEN (100%), RIF (65%)	RIF (20), T/S (2)	E-test	(Torkaman Asadi et al., 2017)
Iran	*B. abortus* (6) *B. melitensis* (24) 2010–2015	DOX (30), TGC (30), CIP (30), STR (30), RIF (30)	T/S (2), TET (9), GEN (5),	E-test	(Irajian et al., 2016)
Iran	*B. melitensis* (18)	DOX (18), TET (18)	STR (2), RIF (15)	Disk diffusion	(Rashidi et al., 2010)
Qatar	*B. melitensis* (231) 2014–2015	DOX, TET, STR, GEN, T/S, CIP (100%)	RIF (48 %)	E-test	(Deshmukh et al., 2015)
Saudi Arabia	*B. melitensis* (704) 1997–2012	TGC (100%). MIC was 0.190-2.0 μg/ml in 36.93%, and $\leq$0.125 μg/ml in 63.07%.	ND	E-test	(Al Johani, 2014)
Saudi Arabia	*B. melitensis* (26) *B. abortus* (1) 1984–1995	TET (100%), STR (100%)	COT (8), RIF (3)	E-test	(Al Shaalan et al., 2002)
Saudi Arabia	*B. melitensis* (63) *B. abortus* (5) 1983–1995	None	COT (40), RIF (5), STR (1), TET (1)	Broth dilution	(Memish et al., 2000)
Saudi Arabia	*B. melitensis* (116)	AZI, GEN, TET, T/S, RIF, CIP, NOR, SPR, TEM (99%–100%)	None		(Qadri et al., 1995)
Saudi Arabia	*B. melitensis* (105)	GEN, RIF, TET, T/S (100%)	Fluoroquinolones (1)	Broth dilution	(Qadri & Ueno, 1993)

(Continues)

TABLE 1 (Continued)

Country	*Brucella* spp. (No.)	Susceptible strains (% or No.)	Non-susceptible strains (% or No.)	Method	References
Saudi Arabia	*B. melitensis* (47)	OFL, DIF, CIP (90%)	None	Broth dilution	(M. Y. Khan et al., 1989)
Turkey	*Brucella* spp. (40) 2000–2013	DOX-RIF (100%), CIP-STR (57.5%), RIF-COT (52.5%), DOX-STR (32.5%), CIP-COT (25%)	RIF highest MIC 1.5 μg/ml	E-test	(Kaysadu et al., 2019)
Turkey	*B. melitensis* (77) 2000–2013	DOX, GEN, CIP, T/S (100%)	CEF (2)	E-test	(Dal et al., 2018)
Turkey	*B. melitensis* (20) 2014–2016	LNZ (100%)	None	E-test, disk diffusion	(Sayiner & Akgun, 2017)
Turkey	*B. melitensis* (50) 2010–2012	DOX, STR, GEN, T/S, CIP, AMP, A/C (100%)	RIF (12)	E-test	(Etiz et al., 2015)
Turkey	*B. melitensis* (80)	RIF, DOX, OFL, LEV, MOX, AZI, CLA (100%)	None	Agar dilution	(Denk et al., 2015)
Turkey	*B. melitensis* (73) *B. abortus* (2) 2009–2011	DOX, TGC, T/S, STR, CIP (100%)	AZI (36) and RIF (34)	E-test	(Parlak et al., 2013)
Turkey	*B. melitensis* (94) 2002–2009	RIF (92)	RIF (2)	E-test	(Sayan et al., 2012)
Turkey	*B. melitensis* (34) 1999–2005	TET, RIF, STR, CIP, DOX, CEF, LEV (100%)	RIF (6)	Microdilution	(Kaya et al., 2012)
Turkey	*B. melitensis* (56) 2008–2009	DOX, STR, T/S, TGC (100%)	None	E-test	(Bayram et al., 2011)
Turkey	*B. melitensis* (76) *B. abortus* (1) 2001–2006	DOX, STR, GEN, T/S, TGC, STR-RIF (100%)	None	E-test	(Ozhak-Baysan et al., 2010)
Turkey	*B. melitensis* (21) 2003–2006	RIF (15)	RIF (6)[a]	E-test	(Sayan et al., 2008)
Turkey	*B. melitensis* (46)	TET, STR, CIP, AZI (100%), RIF (44)	RIF (2)	E-test	(Ayaşlioğlu et al., 2008)
Turkey	*B. melitensis* (60)	TGC, TET, CIP, LEV, MOX (100%)	None	E-test	(Kilic et al., 2008)
Turkey	*B. melitensis* (16) 2004–2005	TGC lowest MIC, TGC-RIF (100%), DOX-RIF (100%)	RIF highest MIC	E-test	(Dizbay et al., 2007)
Turkey	*B. melitensis* (16) 2003	T/S (100%), STR (100%), CIP (93.75%), RIF (93.73%), DOX (75%)		E-test	(Orhan et al., 2005)
Turkey	*B. melitensis* (11) 2002	DOX, RIF, CIP, CEF (100%)	COT (1)	E-test	(Köse et al., 2005)
Turkey	*B. melitensis* (44) 1998–2003	DOX (100%)	None	E-test	(Yamazhan et al., 2005)
Turkey	*B. melitensis* (37) *B. abortus* (5)	DOX, CIP, CEF (42), T/S, (41), RIF (38)	RIF (4), T/S (1)	E-test	(Baykam et al., 2004)
Turkey	*B. melitensis* (41) 2001–2002	T/S, CIP, RIF, CEF, DOX (100%)	None	E-test	(Bodur et al., 2003)
Turkey	*B. melitensis* (43) 1991–1994	AZI, STR, QUI at pH 7.0 RIF, DOX at pH 5.0.		Microdilution	(Akova et al., 1999)

Abbreviations: A/C, amoxycillin/clavulanic acid; A/C, amoxicillin/clavulanic; AMP, ampicillin; AMO, amoxicillin; A/S, ampicillin-sulbactam; AZI, azithromycin; CEF, ceftriaxone; CIP, ciprofloxacin; CLA, clarithromycin; CMP, chloramphenicol; COT, co-Trimoxazole; CLO, cloxacillin; DOX, doxycycline; DIF, difloxacin; ERY, erythromycin; GEN, gentamicin; IMP, imipenem; KAN, kanamycin; LEV, levofloxacin; LIN, lincomycin; LNZ, linezolid; MIC, minimum inhibitory concentration; MOX, moxifloxacin; NOR, norfloxacin; OFL, ofloxacin; OXY, oxytetracycline; RIF, rifampin; SPA, sparofloxacin; SPI, spiramycin; STR, streptomycin; PEN, penicillin; QUI, quinolones; T/S, trimethoprim/sulfamethoxazole; TET, tetracycline; TEM, temafloxacin; TOB, tobramycin; TGC, tigecycline; VAN, vancomycin.
[a]intermediate susceptibility.

TABLE 2 Comparative data of studies on antibiotic therapeutic regimes used for *Brucella* spp. of animal origin in the Middle Eastern and North African (MENA) countries ($n = 13$)

Country	*Brucella* spp. (No.), date	Host	Susceptible antibiotics found	Resistant antibiotics found	Method	References
Algeria	*B. abortus* (11) 2011–2014	Cattle	RIF, GEN, TET, DOX, T/S	STR (4) 36.4%	E-test	(Lounes et al., 2018)
Egypt	*B. abortus* (6) *B. melitensis* (17) 2018–2020	Cattle, sheep, goats	CMP, CIP, DOX, GEN, LEV, STR, TET, T/S, TGC (100%)	AZI (16), RIF (17)	Microdilution, disk diffusion	(Wareth et al., 2021)
Egypt	*B. abortus* (8) *B. melitensis* (21)	Cattle, buffaloes, sheep, goats	CMP, GEN, TET (100%), STR (95%–00%)	CIP (25%–76%), ERY (19%–87%), IMP (25%–76%), RIF (37%–67%)	E-test, PCR WGS	(A. U. Khan et al., 2019)
Iraq	*B. abortus* (4) *B. melitensis* (5)	Animal	STR, GEN, T/S, GEN, RIF, KAN, TET (100%)	DOX, CEF, AMP, ERY	Disk diffusion	(Abbas & Talei, 2010)
Iraq	*B. abortus* (33) *B. melitensis* (25) *B. ovis* (4) 2006–2007	Milk (cow, buffalo, sheep)	STR, GEN, RIF, T/S, KAN (100%), TET (98.5%), DOX (85.9%), CEF (40.8%), AMP (30.9%), ERY (29.5%)	DOX (14.1%), CEF (59.2%), AMP (69.1%), ERY (70.5%)	Disk diffusion	(Abbas & Aldeewan, 2009)
Iran	*B. melitensis* (9)	Sheep, goat	DOX (9), STR, COT, RIF (7)	STR (2), COT (2), RIF (2)	E-test	(Banuo Ashrafganjooyi et al., 2017)
Iran	*B. abortus* (2) *B. melitensis* (36) 2010–2015	Sheep, cow	DOX, CIP, STR, RIF, T/S, TET (38), TGC (34), GEN (36)	TGC (4), GEN (2)	E-test	(Irajian et al., 2016)
Lebanon	*B. abortus* (6) 2004	Dairy products	DOX (5), CEF (5), RIF (4), TET (4), T/S (4)	STR (4), CIP (4), GEN (3), RIF (2), T/S (2)	Disk diffusion	(Alwan et al., 2010)
Syria	*B. melitensis* (100) 2004–2007	Bovine ovine	CIP (97%), DOX (92%), SPA (98%)	STR (100%) RIF (51%), TET (39%)	Broth microdilution	(Al-Mariri & Safi, 2013)
Syria	*B. melitensis* (89) 2004–2007	Animal	TET (89), DOX (89), RIF (78)	RIF (11), STR, SPI, AMO, CEF	E-test	(Safi & Al-Mariri, 2012)
Syria	*B. melitensis* (5)	Animals	TET, RIF, STR, GEN, NOR, CIP, LEV, T/S, AMP, A/C, ERY (5)	None	E-test	(Turkmani et al., 2006)
Turkey	*B. melitensis* (41) 2006–2011	Sheep	RIF (30), STR (38), CIP (38), T/S (20) GEN (38) TET (40), ERY (29), PEN (24), CMP (38), AMP (39), A/C (39), OXY (41), ENR (41)	RIF (11), STR (3), CIP (3), T/S (21), GEN (3), VAN (41), CLO (41), LIN (41), ERY (12)	Disk diffusion	(Ilhan et al., 2013)
United Emirates	*B. melitensis* (30) 2009–2010	Cattle, camel, sheep, goats	CMP, TET, GEN	VAN (2), STR (2), RIF (2), COT (4)	Disk diffusion	(Maymona et al., 2014)

Abbreviations: A/C, amoxycillin/clavulanic acid; A/C, amoxicillin/clavulanic; AMP, ampicillin; AMO, amoxicillin; A/S, ampicillin-sulbactam; AZI, azithromycin; CEF, ceftriaxone; CIP, ciprofloxacin; CLA, clarithromycin; CMP, chloramphenicol; COT, co-Trimoxazole; CLO, cloxacillin; DOX, doxycycline; DIF, difloxacin; ERY, erythromycin; GEN, gentamicin; IMP, imipenem; KAN, kanamycin; LEV, levofloxacin; LIN, lincomycin; LNZ, linezolid; MIC, minimum inhibitory concentration; MOX, moxifloxacin; NOR, norfloxacin; OFL, ofloxacin; OXY, oxytetracycline; RIF, rifampin; SPA, sparofloxacin; SPI, spiramycin; STR, streptomycin; PEN, penicillin; QUI, quinolones; T/S, trimethoprim/sulfamethoxazole; TET, tetracycline; TEM, temafloxacin; TOB, tobramycin; TGC, tigecycline; VAN, vancomycin; WGS, Whole Genome Sequencin.

TABLE 3 Numbers of studies reported resistance on *Brucella* isolates from humans and animals isolates

Antibiotic resistance	Number of studies on human isolates	Number of studies on animal isolates
Resistance to rifampicin	22	8
Resistance to streptomycin	4	7
Resistance to trimethoprim/ sulfamethoxazole	6	3
Resistance to azithromycin	4	1
Resistance to ceftriaxone	4	3

associated with a higher prevalence of brucellosis (Abdelbaset et al., 2018; Dadar et al., 2021; El-Diasty et al., 2022; Samaha et al., 2009). Furthermore, few studies showed that a higher risk of brucellosis is associated with exposure to abortive animals and recently calved animals (Hotez et al., 2012; Musallam et al., 2016; Pappas & Memish, 2007). However, there was no significant connection between brucellosis and pregnancy status, and gender (Abdelbaset et al., 2018).

The control strategies for livestock brucellosis are different from region to region depending on available disease prevalence in each region. It has been well documented that test and slaughter strategy (El-Diasty et al., 2022) and vaccination are among the most effective methods used in controlling and preventing animal brucellosis in endemic regions (El-Diasty et al., 2022; Hou et al., 2019). Four vaccines, S2, RB51, S19, and Rev1, are available to control brucellosis due to *B. abortus* infections in bovids and *B. melitensis* in small ruminants (Hou et al., 2019; Lalsiamthara & Lee, 2017; Zhu et al., 2016). Animal vaccination decreases the prevalence of abortion and protects the remaining animals in the herds. The use of antibiotics in animals is mainly impractical because of the intracellular property of the bacteria in the reproductive organs, lymph nodes, and mammary glands. On the other hand, the recommended optimal treatment for human brucellosis is a combination regimen using two or more antibiotics, including streptomycin, doxycycline, gentamicin, rifampin, or trimethoprim-sulfamethoxazole, to avoid relapses (Solera, 2010; Villate & Casallas, 2020). It has been revealed that monotherapies with single antibiotics and short-term therapy are not proper antibiotic regimens and have been related to the high occurrence of relapse in humans (Alavi & Alavi, 2013). The current treatment of human brucellosis cannot reveal beneficial effects in disease eradication because the relapse rate is approximately 5%−14% despite medication (Jiang et al., 2019; W. Wang et al., 2020; Hasanjani Nimri, 2003; Roushan et al., 2015). Still, eradication of brucellosis is a big challenge and difficult to control. Brucellosis is a true One Health problem that requires multi-sectoral competencies and a transboundary approach to increase awareness from consumers to decision-makers in this region (Bagheri Nejad et al., 2020).

3.3 | Early trials for the treatment of brucellosis globally

David Bruce carried out the first trials for the treatment of brucellosis in humans in 1889 (Bruce, 1889). The author has reported that there were no effective drugs for the treatment of Malta fever. Many physicians applied rectal administration of 'quinine' and stated that it lowered the temperature and shortened the clinical disease. Bruce also tested the oral and per rectum drug on several patients (Bruce, 1889). Different authors have tried 'sulphanilamide' as a potential treatment of human brucellosis (Francis, 1938). However, it failed to cure a case of brucellosis with endocarditis (Smith & Curtis, 1939). In 1947, 'streptomycin' was used by Pulaski and Amspacher, and they found that *B. melitensis* was susceptible to streptomycin (Pulaski & Amspacher, 1947). Streptomycin was tested on 17 brucellosis cases (10 with chronic and seven with acute brucellosis) with different application times and variable dosages. The authors reported that 10 days of daily administration of streptomycin in a dosage of l–2 g revealed no beneficial effect. However, long application time with larger doses did show some effect. The 'sulphadiazine' was used to treat patients with outstanding results, although the occurrence of relapses was also very common (Spink et al., 1949). The 'chlortetracycline' was the first member of tetracyclines discovered in 1945. The trade name is Aureomycin, and it showed efficient action against a wide range of microorganisms. However, treatment trials were not done until 1948, when Spink and coworkers used the drug to treat human brucellosis in Mexico (Spink et al., 1948). The authors treated 24 patients who proved to be infected with *B. melitensis* through haemoculture. Sixteen patients were treated with a combination of aureomycin and sulphadiazine for 9–13 days and showed promising results, while the other eight patients received aureomycin only. Aureomycin was given to the patients in a gradually increasing dose with a maximum of 2 g per day for 11 days to avoid side effects. The fever was cured within 2–3 days (Spink et al., 1948).

The combination of 'dihydrostreptomycin sulfate' and chlortetracycline was used to treat human brucellosis for the first time in 1950. Twenty-five patients with positive blood cultures were treated, and this combination was effective (Herrell & Barber, 1950). In 1952, the same authors used a combination of 'oxytetracycline' with 'dihydrostreptomycin' to treat 35 patients. Results showed that both combinations (dihydrostreptomycin sulfate/ chlortetracycline and oxytetracycline/dihydrostreptomycin) were equally effective, and only 2/60 patients in both groups had relapses (Herrell & Barber, 1952). 'Rifampicin' was applied with other antibiotics in an in-vitro experiment to determine the susceptibility of *Brucella* organisms to different antibiotics (Hall & Manion, 1970). The authors found that rifampicin was one of the most effective drugs against *Brucella* and inhibited 50% of *Brucella* strains at a minimum inhibitory concentration (MIC) level of 0.3 μg/ml. Although rifampicin is proven to be effective when used alone, the authors did not recommend its use alone because the detection of resistant strains was reported (Stuart, 1982). 'Trimethoprim/sulfamethoxazole' is known as 'co-trimoxazole' and was used alone to treat 86 patients in a dose of two tablets twice daily for a

month, each tablet contained 80 mg of trimethoprim plus 400 mg of sulfamethoxazole. Blood cultures were taken every 15 days during the treatment. It was reported that 78 of 86 patients showed a good clinical response and the symptoms disappeared. While the remaining eight patients did not respond satisfactorily and clinical signs did not cure completely, 4% relapse was seen after 2 months among all patients (Daikos et al., 1973). It is worth mentioning that a relapse rate among 40% of patients treated with co-trimoxazole alone was also reported (Ariza et al., 1986).

3.4 | Current knowledge on antibiotic susceptibility of *Brucella* spp. strains from humans in the MENA region

Despite the high prevalence of human brucellosis in the Middle Eastern countries such as Egypt, Syria, Iraq, Saudi Arabia, and Iran and frequent treatment, isolates in several studies have remained in-vitro susceptible to the majority of the tested antibiotics such as rifampin, ceftriaxone, doxycycline, streptomycin, trimethoprim-sulfamethoxazole, and gentamicin. Only 40 studies have been published on antibiotic susceptibility and therapeutic regimes using *Brucella* spp. isolates from humans in the MENA countries. Almost all studies investigated *B. melitensis*, while *B. abortus* was investigated only in eight of these studies (Table 1).

3.4.1 | Rifampicin

Rifampicin is also known as rifampin. It is a bactericidal drug that can kill intracellular bacteria by inhibiting bacterial RNA synthesis. It is a derivative of rifamycin B and was used in clinical trials for the first time in 1967 (Konno et al., 1973). Rifampin is an essential and effective antibiotic in the treatment of brucellosis, and it is extensively recommended for first-line therapy (Ariza et al., 1992; Taghvaee et al., 2011). It has sound bacteriostatic or bactericidal effects with ideal intracellular penetration and evident synergism along with other antibiotics. Thus, such combinations have been proposed by WHO to manage brucellosis (Sandalakis et al., 2012). However, several reports describing probable resistance to rifampin in brucellosis were published, for example, from Egypt (Abdel-Maksoud et al., 2012; Wareth et al., 2021), Iran (Alamian et al., 2019; Farazi et al., 2018; Razzaghi et al., 2016), Turkey (Baykam et al., 2004; Etiz et al., 2015; Kaya et al., 2012), Saudi Arabia (Al Shaalan et al., 2002), and Qatar (Deshmukh et al., 2015). Indeed, more than half of the studies (22/40) that were carried out on human isolates reported rifampicin resistance. This resistance to rifampicin can be explained by the similar treatment schemes of brucellosis and tuberculosis in the Middle East region (Ariza et al., 2007). Additionally, it has been reported that rifampicin did not yield good results in some cases of brucellosis in adults in Iran (Roushan et al., 2004). Possible rifampicin resistance was reported in some *B. melitensis* isolates from Egypt between1999 and 2007; however, the molecular basis of the resistance has not been investigated (Abdel-Maksoud et al., 2012). Nevertheless, few molecular-based investigations have evaluated the genetic basis of decreased susceptibility or resistance to certain antibiotics. Mutations in the *rpo*B gene of rifampicin-resistant *B. melitensis* have been reported in at least three studies (Bazrgari et al., 2020; Khan et al., 2019; Sayan et al., 2008). Moreover, the combination of rifampicin and doxycycline may also pose problems in many developing countries, including those of the Middle Eastern, because this combination may cause rifampicin resistance in other agents, especially in *Mycobacterium tuberculosis* (del Pozo & Solera, 2012).

3.4.2 | Tetracyclines

Tetracyclines were discovered in 1944 as bacteriostatic drugs that were able to inhibit bacterial protein synthesis. The substance chlortetracycline was followed by oxytetracycline, tetracycline, metacycline, doxycycline, and minocycline (Chopra & Roberts, 2001). Their antibacterial activities are similar, except for doxycycline, which has double activity as tetracycline. The average MIC of all tetracyclines for *Brucella* isolates was reported at $<1\ \mu$g/ml (Farrell et al., 1976). Doxycycline is a semi-synthetic derivative of oxytetracycline and is highly lipid-soluble, resulting in higher intracellular penetration and better tissue distribution than other tetracyclines. A synergistic effect was seen in combining tetracyclines with streptomycin or rifampicin on intracellular *Brucella* organisms (Farrell & Robertson, 1980). Hence, the majority of MIC studies performed in Middle Eastern countries with endemic brucellosis showed that the *Brucella* spp. isolates have maintained their susceptibility to doxycycline (Abdel-Maksoud et al., 2012; Alamian et al., 2019; Asadi et al., 2016; Baykam et al., 2004; Bayram et al., 2011; Deshmukh et al., 2015; Ilhan et al., 2013; Qadri & Ueno, 1993; Razzaghi et al., 2016; Turkmani et al., 2006; Table 1).

3.4.3 | Aminoglycosides

Aminoglycosides are bactericidal compounds that interfere with bacterial protein synthesis. Streptomycin was discovered in 1944 as the first compound of the aminoglycosides, followed by gentamicin, kanamycin, amikacin, dihydrostreptomycin, neomycin, tobramycin, sisomicin, and netilmicin. Only three of these antibiotics have been used to treat brucellosis, that are, streptomycin, gentamicin, and netilmicin (Meng et al., 2018). Streptomycin alone is ineffective in treating brucellosis; however, the synergistic effect of its combination with tetracyclines is well documented. More than half of studies (7/13) that were carried out on animal isolates reported resistant isolates to streptomycin, while in human isolates, resistance was reported only in two studies from Iran (Farazi et al., 2018; Rashidi et al., 2010) and one from Saudi Arabia (Memish et al., 2000). Streptomycin is known as one of the most effective compounds in the treatment of human brucellosis (Bayindir et al., 2003). A combination of streptomycin with rifampicin and doxycycline is proposed for the treatment of patients suffering from spondylitis (Trott et al., 2018). In a study of 160 patients with brucellosis in Saudi Arabia, resistance to streptomycin was present in 0.6% of the

isolates studied, while 29% showed resistance to sulfamethoxazole-trimethoprim, 3.5% to rifampicin, and 0.6% to doxycycline (Memish et al., 2000).

Gentamicin has the same mode of action as streptomycin; however, nephrotoxicity is more often reported with gentamicin. It was regularly used in patients with *Brucella* endocarditis (Houang & Greenwood, 1977). The MIC of gentamicin for brucellae was estimated at 2 μg/ml. Only one article reported resistance to gentamycin in human isolates from Iran (Irajian et al., 2016) and two in animal isolates from Turkey and Lebanon (Alwan et al., 2010; Ilhan et al., 2013). Netilmicin is the newest member of the aminoglycosides. It is a derivative of sisomicin with a half life span of 2–2.5 h in adults, and 4–18 h in newborn and premature babies (Scholar, 2007). It is also known to be active against gentamicin-resistant Gram-negative bacilli. A single daily dose (SDD) of netilmicin was used to treat active brucellosis. It was administrated for 7 days in a combination with doxycycline for the treatment of 64 patients. The combination was safe, and no nephrotoxicity or ototoxicity has been detected among patients. However, the therapeutic failure was seen in five (18%) patients, and relapses occurred in eight (12.5%) patients (Solera et al., 1996).

It seems that *Brucella* isolates from the Middle East region were regularly susceptible to streptomycin (Abdel-Maksoud et al., 2012; Turkmani et al., 2006), except for those from Iran (Farazi et al., 2018; Rashidi et al., 2010) and Saudi Arabia (Memish et al., 2000). *Brucella* isolates were also regularly susceptible to gentamicin (Abed Mohamad, 1998; Alamian et al., 2019; Deshmukh et al., 2015; Etiz et al., 2015; Farazi et al., 2018; Wareth et al., 2021). However, the use of any of the three aminoglycosides (streptomycin, gentamicin, or netilmicin) for less than 2–3 weeks was resulted in a higher therapeutic failure (Solera et al., 1996).

3.4.4 | Sulfonamides

Co-trimoxazole is a combination of trimethoprim (TMP) and sulfamethoxazole (SMZ) in a ratio of 1:5. Both compounds act by interrupting the synthesis of bacterial purine but at different levels (Salter, 1982). TMP is a bactericidal drug but is more effective when combined with SMZ (Bushby & Hitchings, 1968). Thus, TMP-SMZ combinations display synergistic effects on intracellular bacteria and are recommended for brucellosis treatment. It should be used in combination with rifampin in children under 8 years and pregnant women and combined with doxycycline and rifampin in treating *Brucella*-induced endocarditis (Maves et al., 2011). Different authors report that TMP-SMZ is an effective antibiotic with low MIC levels (Abdel-Maksoud et al., 2012; Alamian et al., 2019; Etiz et al., 2015). It was also found to be the most effective antimicrobial agent in treating human brucellosis (Parlak et al., 2013). However, six reports on human isolates showed a decreased susceptibility to TMP-SMZ, one from Crete (Turkmani et al., 2006), one from Turkey (Baykam et al., 2004), and four from Iran (Farazi et al., 2018; Hashemi et al., 2016; Irajian et al., 2016; Torkaman Asadi et al., 2017). A higher rate of resistance to sulfamethoxazole-trimethoprim has been noticed in Saudi Arabia (Almuneef et al., 2003; Bannatyne et al., 2001). However, no relapse was noticed when TMP-SMZ is used in combination with rifampicin with or without streptomycin (Al Shaalan et al., 2002).

3.4.5 | Fluoroquinolones

Fluoroquinolones are a group of broad-spectrum bactericidal antibiotics that interfere with bacterial DNA synthesis (Majalekar & Shirote, 2020). Their in-vitro activity against *B. melitensis* has been reported. However, they are not efficient as monotherapy against active brucellosis (Khan et al., 1989; Lang & Rubinstein, 1992). The group includes ciprofloxacin, norfloxacin, lomefloxacin, ofloxacin, clinafloxacin, pefloxacin, sparofloxacin, difloxacin, and fleroxacin. The fluoroquinolones are associated with a high unacceptable therapeutic failure and relapses, development of resistance, and failure to develop in-vitro synergy with other antibiotics (Akova et al., 1993). The combinations of ofloxacin-rifampicin versus doxycycline-rifampicin were tested in 61 patients with active brucellosis. One case of failure and one relapse case were seen in the ofloxacin-rifampicin group out of 31 patients. In contrast, in the doxycycline-rifampicin group, one relapse out of 30 patients has been reported. Thus, fluoroquinolones should not be used as first-line therapy in humans with active brucellosis (Akova et al., 1993). The higher and lower MIC values for ciprofloxacin have been reported previously. The effectiveness of ciprofloxacin in the treatment of brucellosis is discussed controversially (Abdel-Maksoud et al., 2012; Al Dahouk et al., 2005). Resistance to this antibiotic has been reported in Iran (Farazi et al., 2018) and Saudi Arabia (Qadri & Ueno, 1993).

3.4.6 | β-Lactam antibiotics

Cephalosporins, particularly the third-generation group, have broad efficacy against Gram-negative organisms by inhibiting the mucopeptide synthesis in the bacterial cell wall (Akova et al., 1999). Although MICs ranged from 0.25 to 2 μg/ml of ceftriaxone and it was in-vitro effective against *B. melitensis* (Palenque et al., 1986), a high incidence of therapeutic failure was noted in patients with active brucellosis (Lang & Rubinstein, 1992). In Kuwait, ceftriaxone was administrated to treat 14 adults with active brucellosis. Nine patients (64.3%) responded to treatment, but treatment failure was reported in five (35.7%) patients (Al-Idrissi et al., 1989). Reduced susceptibility to ceftriaxone was also seen in cases of human brucellosis from Iran (Farazi et al., 2018), Turkey (Dal et al., 2018), and Egypt (Abdel-Maksoud et al., 2012). A study evaluated the use of ceftriaxone in a small number of patients ($n = 14$) with brucellosis in Saudi Arabia, reported treatment failure in 30.8% of patients; while another study in Saudi Arabia reported the efficacy of ceftriaxone in treatment of complicated conditions of brucellosis such as neurobrucellosis and orchitis (Fatani et al., 2019). Despite these promising results, further studies are needed before considering ceftriaxone as a first-line therapy of human brucellosis. Probable resistance to ampicillin-sulbactam and penicillin has been reported from

human isolates of *Brucella* spp. in Iran (Alamian et al., 2019) and Iraq (Abed Mohamad, 1998). Amoxycillin has been used alone or in combination with gentamicin and rifampicin and showed variable degrees of response. Amoxycillin was used to treat 28 patients with acute brucellosis. All patients responded very well to treatment, but seven patients (25%) showed clinical and microbiological evidence of relapse (Papapolizos et al., 1980).

3.4.7 | Macrolide and chloramphenicol antibiotics

Azithromycin is one of the macrolides characterized by its rapid distribution after oral administration with higher concentrations within cells, particularly phagocytes (Drew & Gallis, 1992). Its in-vitro activity against *B. melitensis* was tested in comparison to tetracycline, and it was able to cure murine brucellosis experimentally infected with *B. melitensis* (Lang et al., 1994). A study from Spain revealed a slight difference in the sensitivity of *B. melitensis* to azithromycin and tetracycline, indicating a promising therapeutic role of azithromycin in human brucellosis (Landinez et al., 1992). Four studies on human isolates and one on animal isolates have reported resistance to azithromycin. In Saudi Arabia, in-vitro activity of azithromycin was investigated among 116 *B. melitensis* isolates from 115 patients. All isolates were inhibited by 2.0 μg/ml of azithromycin, while 87% of isolates were inhibited by 1.0 μg/ml (Qadri et al., 1995). Despite its in-vitro activity, clinical trials to determine its effectiveness in treating brucellosis were not done. Three studies showed a decreased susceptibility to azithromycin in isolates from Turkey (Parlak et al., 2013), Iran (Farazi et al., 2018), and Egypt (Wareth et al., 2021). A regimen of azithromycin plus gentamicin resulted in a high rate of relapses and therapeutic failures (30%–50%) among 10 patients (Solera et al., 2001). Chloramphenicol was used to treat brucellosis in the 1950s (Knight et al., 1950). However, it has not been used in recent years due to severe side effects, particularly bone marrow toxicity and suppression, and thus it is not recommended to treat human brucellosis (Knight et al., 1950).

3.5 | Current knowledge on antibiotic susceptibility of animal *Brucella* isolates in MENA region

Few studies ($n = 13$) have been performed on antimicrobial susceptibility testing of *Brucella* spp. isolates from animals in MENA countries. The studies were conducted on isolates from eight countries; 11 studies included *B. melitensis* isolates, while *B. abortus* was investigated in seven studies. Only one study from Iraq included four isolates of *B. ovis* (Abbas & Aldeewan, 2009). Resistance to rifampicin, streptomycin, trimethoprim/sulfamethoxazole, ceftriaxone, and azithromycin was reported in eight studies, seven studies, three studies, three studies, and one study, respectively (Table 2). In Egypt, a study undertaken on 29 *B. abortus* and *B. melitensis* isolates showed 100% susceptibility to chloramphenicol, gentamicin, tetracycline, and 95%–100% to streptomycin using E-test (Khan et al., 2019). Resistance to ciprofloxacin (25%–76%), erythromycin (19%–87%), imipenem (25%–76%), and rifampicin (37%–67%) has been found in *B. abortus* and *B. melitensis* isolates. The study showed that rifampicin resistance was associated with mutations in the *rpo*B gene in all phenotypically resistant isolates. Furthermore, resistance to ciprofloxacin was associated with mutations in *gyr*B and *gyr*A genes in four phenotypically resistant isolates of *B. melitensis* (Khan et al., 2019). Recently, susceptibility testing was carried out on 23 isolates from different animal hosts by microdilution and disk diffusion tests. At least, 16 isolates showed non-susceptibility (resistant and intermediate) to rifampicin and azithromycin; however, all isolates were still in-vitro susceptible to the majority of antibiotics used in human treatment, for example, doxycycline, tetracyclines, gentamicin, ciprofloxacin, levofloxacin, chloramphenicol, streptomycin, trimethoprim/sulfamethoxazole, and tigecycline (Wareth et al., 2021). Whole genome sequencing of resistant isolates revealed the absence of classical AMR genes. In Iraq, resistance to doxycycline, cephalexin, cefotaxime, ampicillin, and erythromycin was identified in a study of 62 isolates from cows and buffalos milk, and sheep (Abbas & Aldeewan, 2009). However, resistance to rifampicin, streptomycin, and trimethoprim/sulfamethoxazole was seen in *B. melitensis* strains recovered from sheep and goats (Banuo Ashrafganjooyi et al., 2017).

In a study from Iran, the antimicrobial susceptibility of nine isolates of *B. melitensis* from raw milk of nomadic livestock showed 100% sensitivity to doxycycline. However, two isolates were found resistant to streptomycin, cotrimoxazole, and rifampin. None of these *Brucella* isolates has been subjected to molecular investigation for mutations of the *rpoB* gene or other genes such as *gyr*B and *gyr*A (Banuo Ashrafganjooyi et al., 2017). Another Iranian study also confirmed that most *Brucella* isolates from animals are susceptible to trimethoprim-sulfamethoxazole, doxycycline, rifampin, ofloxacin, and ciprofloxacin, but are increasingly resistant to tigecycline (Irajian et al., 2016). Different *B. melitensis* isolates from the livestock of Abu Dhabi Emirate also highlighted the variable percentages of sensitivity to vancomycin, doxycycline, streptomycin, cotrimoxazole, chloramphenicol, rifampin, tetracycline, and gentamycin, while two isolates were found resistant to vancomycin, streptomycin and rifampin, and four to cotrimoxazole as well (Maymona et al., 2014). Antimicrobial susceptibility testing of 41 Turkish *B. melitensis* isolates from sheep showed resistance to streptomycin, ciprofloxacin, and gentamicin. The highest resistance (100%) was determined against vancomycin, cloxacillin, and lincomycin, followed by trimethoprim/sulfamethoxazole (50%). However, all strains were reported to be sensitive to oxytetracycline, enrofloxacin, and tetracycline. Thus, ovine *B. melitensis* strains were resistant to at least one antimicrobial (Ilhan et al., 2013).

Three studies have been carried out on isolates from domestic animals and animal products from different Syrian regions. Ciprofloxacin, doxycycline, and oxytetracycline were the most effective antibiotics. Resistance to rifampin (51%), streptomycin (100%), and tetracycline (39%) was seen in 100 *B. melitensis* isolated between 2004 and 2007 (Safi & Al-Mariri, 2012). In addition, 23 *B. melitensis* strains from Mediterranean (Israel and Turkey) and African countries were analyzed in Norway (Johansen et al., 2018). All strains were sensitive to all tested antibiotic compounds except for rifampicin. Despite all strains

were phenotypically resistant or intermediate to rifampicin using broth microdilution and gradient strip methods, no mutations were found in the *rpoB* gene (Johansen et al., 2018). Therefore, resistance based on phenotyping was overestimated.

3.6 | Gaps in implementation of antibiotic therapeutic strategies in *Brucella*

Regular antibiotic susceptibility testing is not commonly recommended in brucellae due to the high risk to laboratory personnel, their overall susceptibility to many antimicrobial agents used in combination for treatment, and the fastidious nature of the organisms (Lonsway et al., 2010). Besides, there are no precise breakpoints for brucellae, and EUCAST and CLSI have not validated several antibiotics. There are still several obstacles to overcoming human brucellosis, including the difficulty of patients follow-up in rural areas, disappearance of symptoms after initial treatment, the risk of developing of resistance to rifampicin in countries with endemic tuberculosis and relapses, which affect approximately 5%—16% of brucellosis patients (Ariza et al., 1992; del Pozo & Solera, 2012; Pappas & Memish, 2007). Treatment of brucellosis therapy may be complicated by osteoarticular infections, neurobrucellosis, or endocarditis (Ma et al., 2021). These forms of localized infections may require a more aggressive or more prolonged therapy than uncomplicated brucellosis. Active brucellosis should be treated with care, as tetracyclines are contraindicated, and aminoglycosides should be used only when monitoring of serum concentration levels is available. Co-trimoxazole and rifampicin are associated with resistance and high rates of relapse. Tetracyclines are contraindicated for children below 7 years due to side effects on bone and teeth (Shutter & Akhondi, 2021). Nursing mothers should avoid tetracyclines because excretion in breast milk might occur. Currently, no data on the effects of tetracyclines excreted in breast milk on children are available (Vojtová & Urbánek, 2009). Doxycycline is less likely to affect teeth and bone growth and is recommended for treating brucellosis. However, in poor endemic countries, cheap alternative forms of tetracyclines that are commonly manufactured locally can be used. Although fluoroquinolones are successful drugs in brucellosis treatment due to the good penetration properties at the intracellular level, the application of fluoroquinolones alone causes frequent relapses reach to 21%—66% (Acocella et al., 1989; Castillo et al., 1989; Falagas & Bliziotis, 2006). Therefore, it should not be used as a monotherapy. It is difficult to decide whether active brucellosis after treatment is a relapse or a reinfection. Nevertheless, evidence of active brucellosis in the form of reappearance of signs, increased antibodies titres, or positive blood culture will require immediate treatment (Madkour, 2001).

4 | DISCUSSION

Brucellosis remains an endemic disease in MENA countries. In the MENA region, the incidence of brucellosis is increasing, and new hot spots continue to appear occasionally (Musallam et al., 2016; X. H. Wang & Jiang, 2020). Human brucellosis is endemic in different parts of the world, including the Middle East, and is commonly caused by *B. melitensis*. Antibiotic therapy is the primary approach for the complete eradication of the microorganism despite the intracellular lifestyle of the pathogen that leads to relapses in 5%–14% of patients after treatment (del Pozo & Solera, 2012; Skalsky et al., 2008). Therefore, combined therapy is recommended in human brucellosis treatment. The most commonly used combinations are doxycycline-gentamicin and doxycycline-streptomycin (del Pozo & Solera, 2012). Rifampicin combined with quinolones was significantly less efficient in treating brucellosis patients than doxycycline combined with streptomycin or rifampicin (Skalsky et al., 2008). Therefore, WHO recommended a 6-week course of combination therapy with rifampicin-doxycycline (Ariza et al., 1992).

Few trials were carried out for treating infected farm animals using high doses of antibiotics. However, most of these trials lacked the tools to ensure complete eradication of brucellae, and many treated animals relapsed (Radwan et al., 1993; Radwan et al., 1992, 1995, 1989). In specific conditions, chloramphenicol, streptomycin, sulphadiazine, and chlortetracycline have been proposed for the imperative treatment of animals (Pal et al., 2020). However, antibiotics therapy is not economically feasible in livestock and appropriate antibiotic therapy is still ambiguously discussed (Mohan & Saxena, 2020; Prajapati et al., 2014), and should be restricted to a few situations, for example endangered livestock species, exceptional breeding animals. There has been vast scientific progress on genomic sequence analysis of brucellae over the last few years, and the virtual absence of classical antimicrobial resistance determinants in the genome of brucellae is puzzling (Wareth et al., 2021). There are many factors responsible for knowledge gap in the effective treatment of brucellosis. Among these factors are the nature of brucellae as intracellular organisms which effectively protect them from antibiotics.

Antibiotic regimens for human brucellosis are facing many obstacles, namely, relapses, therapeutic failure, reinfection, and the eruption of resistant *Brucella* strains, in addition to side effects of current antibiotics (Franco, Mulder, & Smits, 2007). Moreover, special care should be taken in treating chronic patients suffering from localized infections that require high doses of antibiotics and more extended administration times. Furthermore, the development of antibiotics by researchers and pharmaceutical companies was not primarily directed to the treatment of brucellosis. There are other unwanted side effects of antibiotic treatment, for example adverse effects in children, troublesome parental administration of aminoglycosides, need for long-term treatment, or low therapeutic efficacy of antibiotics (Alavi & Alavi, 2013; S. Khan et al., 2020). The treatment of brucellosis could also lead to the emergence of multidrug resistance (Alavi & Alavi, 2013). Different methods have been used to determine MIC for *Brucella*, including broth microdilution, broth macrodilution, agar dilution, and E-test strips (Trott et al., 2018), but phenotypic in-vitro resistance is sometimes not linked to therapeutic success. The slow-growing nature of *Brucella* organisms is considered an impediment for using current standard procedures to determine the minimum inhibitory concentration (MIC) of antibiotics. To overcome this obstacle, the Clinical and

Laboratory Standards Institute recommended inoculating *Brucella* medium (pH 7.1) with a 0.5 McFarland standard inoculum and incubation at 35 ± 2°C in an aerobic atmosphere for 48 h before reading MICs.

Although different studies in the Middle Eastern countries showed that *Brucella* isolates are commonly susceptible to many antibiotics, sporadic cases of disease relapse and antibiotic resistance have been documented (El Ariza et al., 1986; Miedany et al., 2003). On the other hand, fastidious growth requirements, the need for a biological safety level 2 or 3 laboratories, and the risk of laboratory-acquired infections led to the failure to perform antimicrobial susceptibility testing for the brucellae from clinical specimens (Abdel-Maksoud et al., 2012). The persistence of clinical signs after treatment is considered a therapeutic failure (Abramson et al., 1997), and there is no consensus regarding the duration of symptoms persistence after starting treatment (Cisneros et al., 1990). Duration of treatment is variable from one study to another, and differentiation between relapses and reinfection is a big challenge in endemic areas where patients are continually exposed to the risk of reinfection. Considering the differences of brucellosis pathophysiology between humans and animals, studies suggested the combination of doxycycline with streptomycin, gentamicin, or rifampin for a duration ranging from 1 to 6 weeks according to the selected combination, and application of combinations with TMP-SMX is considered in cases of pregnancy (Bosilkovski et al., 2021; Pappas et al., 2006).

5 | CONCLUSIONS

Brucellosis is an ancient zoonotic disease that is associated with the consumption of contaminated raw milk and dairy products. Therapeutic regimes for brucellosis rely mostly on non-blinded, non-randomized, and uncontrolled studies. The antimicrobial resistance of *Brucella* spp. is growing in Middle Eastern countries, where the disease is notoriously endemic. It is not surprising that relapses following brucellosis therapy do occur due to the intracellular lifestyle of brucellae in reticuloendothelial cells, the tendency to become chronic in humans, and their ability to use 'niches' such as bones that only a few antimicrobial classes can reach. On the other hand, relapses are caused by the development of resistant bacteria because of the application of inappropriate doses, inappropriate substances, or simply insufficient duration of therapy. Finally, choosing the best antimicrobial compounds and course of treatment remain the main critical factors for successful treatment.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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