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In Vitro Antimicrobial Susceptibility of *Brucella melitensis* Isolates from Sheep in an Area Endemic for Human Brucellosis in Turkey

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ABSTRACT. The aim of this study was to assess *in vitro* antimicrobial susceptibility of *Brucella melitensis* isolates isolated from naturally infected sheep cases in an area where human brucellosis is endemic, focusing on rifampin (RIF), streptomycin (SM), ciprofloxacin (CPFX), trimethoprim/sulfamethoxazole (TMP/SMZ), gentamicin (GM) and tetracycline (TC) and on 11 other antimicrobials. The identification and typing of *Brucella* isolates were carried out using standard classification tests and polymerase chain reaction (PCR) methods. Antimicrobial susceptibility testing was carried out on Mueller-Hilton agar. The resistance to SM, CPFX and GM was determined at the rate of 7.3% and to RIF at the rate of 9.7%. The highest (46.3%) resistance was determined against TMP/SMZ. All strains were found to be sensitive to TC at the rate of 100.0%. In conclusion, ovine origin *B. melitensis* strains evaluated in this study were resistant to at least one antimicrobial (51.2%) that is commonly used in human clinical medicine against brucellosis.
KEY WORDS: antimicrobial susceptibility, *Brucella melitensis,* sheep, zoonosis.

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Brucellosis is a zoonosis that has serious implications for domesticated animals and humans caused by members of genus *Brucella*. Brucella species can infect cattle, sheep, goats, camels, dogs and pigs, and it can cause abortions, the birth of sickly offspring, retained placenta, orchitis, epididymitis and loss of milk and meat production in animals [31, 37].

Brucella melitensis (B. melitensis) is normally associated with infection in small ruminants, but other species, including dogs, cattle and camels, can be infected. In some countries, particularly in the Middle East, *B. melitensis* infection of cattle has emerged as an important problem [6, 30, 37]. This agent remains the major cause of human brucellosis worldwide, followed by *B. abortus* and *B. suis*; while rare, persisting cases of *B. canis* human infection and disease by novel *Brucella* pathogens of marine mammals have also emerged [6, 30].

People contract the disease by direct contact with contaminated fetal membranes or more commonly, as a result of the consumption of contaminated unpasteurized milk and milk products [37, 39]. Human brucellosis is a multisystemic disease and is the commonest zoonotic infection worldwide [30, 37]. It is endemic in the Mediterranean region, the Middle East, Central Asia and parts of Africa and Latin America [6, 14, 30, 37].

Human brucellosis is endemic in Turkey, and the incidence is estimated to be 0.59 per 100,000 per year, but can be much higher among specific groups. The high incidence of brucellosis in Turkey is due to the widespread infection among domestic animals and the frequent contacts with livestock and the consumption of raw milk and traditionally prepared soft cheeses by the rural population [1]. So, brucellosis and its complications are still a serious public health concern, especially in Eastern Anatolia of Turkey [1, 13, 18, 36]. In the region of Van (located in Eastern Anatolia), which is an area of intensive animal husbandry, brucellosis is common in sheep [16, 17].

The essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics, such as tetracycline (TC), doxycycline (DOXY), streptomycin (SM), rifampin (RIF), gentamicin (GM), trimethoprim/sulfamethoxazole (TMP/SMZ), fluoroquinolones and aminoglycosides, for an adequate length of time [6, 14, 37]. The guidelines of the World Health Organization (WHO) [37], last published in 1986, recommended DOXY with RIF for six weeks in place of their previously recommended regimen of TC for six weeks in combination with SM for the first two to three weeks. During the following years, a number of clinical studies assessed the efficacy of different regimens, and the reports from various regions of the world revealed that the WHO-recommended regimens have not been universally applied in clinical practice. So, the relative merits of these two regimens are still being discussed [6, 14, 15, 37].

Some studies connecting the *in vitro* antimicrobial susceptibility of *Brucella* isolates isolated from humans were performed [4, 10, 20, 27]. However, a few studies have been performed to detect the treatment regimens in animal brucellosis [32, 33]. To our current knowledge, *in vitro* antimicrobial susceptibility of *Brucella* isolates isolated from animals to antimicrobial agents that are commonly used as basic or alternative antimicrobials in human brucellosis has not been reported. In order to contribute to the treatment of human

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brucellosis, in this study, we aimed to assess the *in vitro* antimicrobial susceptibility of *B. melitensis* isolates isolated from naturally infected sheep cases in an area that human brucellosis is endemic, focusing on RIF, SM, CPFX, TMP/SMZ, GM and TC and on 11 other antimicrobials.

MATERIALS AND METHODS

Samples: Samples were collected from aborting ewes in the region of Van (Eastern Anatolia, Turkey) where human brucellosis is endemic. Swabs from the pharynx of aborted ewes fetuses and milk samples from the aborted ewes were obtained and used as samples. These samples were collected from the 117 different sheep flocks. The total sheep number in these flocks was 13,629, and there were 1,387 aborted ewes in these flocks. Of the sheep aged between 3 and 7 years old, none of the animals were previously immunized against *Brucella.* There was a family history of brucellosis in 14.5% of the cases.

Brucella isolates: A total of 41 *Brucella* isolates from naturally infected sheep cases (32 from fetuses and 9 from milk samples) isolated between 2006 and 2011 were studied. Of them, 23 (56.1%) isolates were isolated from the owners that were treatment of brucellosis before. All isolates were stored at −70°C and subcultured twice before the susceptibility test.

Identification methods: Swabs were immediately inoculated onto duplicate plates of blood agar (1.10886, Merck, Darmstadt, Germany) supplemented with 5% defibrinated sheep blood (v/v) and *Brucella* selective supplement (SR083A) (Oxoid, Hampshire, U.K.). Milk samples were plated onto duplicate plates of Farrell's modified serum dextrose agar. Farrell's modified serum dextrose agar was prepared from blood agar base no. 2 (CM271, Oxoid) supplemented with *Brucella* selective supplement, 7% horse serum and 1% glucose. All plates were incubated at 37°C, both in air and microaerobically (5–10 CO_2%) for 5–7 days. *Brucella* isolates were identified using standard classification tests including colony morphology, Gram stain, catalase, oxidase and urease activities, H_2S production, lysis by Tbilisi (Tb) phage, CO_2 requirement, dye inhibition tests with thionine (40, 20 and 10 mg/m*l*) and basic fuchsine (20 and 10 mg/m*l*) and the slide agglutination test with monospecific A and M sera [3, 31].

Polymerase chain reaction (PCR): For the identification of the *Brucella* isolates, PCR assay was also applied. Briefly, a modification of the method described by Leal-Klevezas *et al.* [21] was used for the extraction of *Brucella* DNA from the isolated colonies. The PCR protocol and *B. melitensis*-specific primers used were previously described by Bricker and Halling [11]. The sequences of the primers were 5'-AAA, TCG, CGT, CCT, TGC, TGG, TCT, GA-3' (*B. melitensis*-specific primer) and 5'-TGC, CGA, TCA, CTT, AAG, GGC, CTT, CAT-3' (IS711-specific primer). The temperature cycling for the amplification was performed in a DNA thermalcycler (Thermo Electron Corp., Waltham, MA, U.S.A.). The amplified products were separated in a 1.5% (w/v) agarose gel containing 1× TBE buffer, stained with ethidium bromide (0.5 μg/m*l*) and evaluated by a computerized image analysis system (Spectronics Co., Westburg, NY, U.S.A.). A visible band of appropriate size (731 bp) was considered as a positive reaction for *B. melitensis*. A positive control (based on DNA from *B. melitensis* 16 M reference strain) and a negative control (DNase and RNase free water) (A2864, Applichem, Darmstadt, Germany) were included in all the tests. To check the reliability of the results and to detect any external contamination, all samples were processed in duplicate.

Antimicrobial susceptibility testing: We applied the Kirby-Bauer disc diffusion method [8] to test isolates for sensitivities to selected antibiotics. The Mueller-Hinton agar (CM337, Oxoid) supplemented with 5% defibrined sheep blood was used. A suspension of the cultures was prepared in sterile saline solution (NaCl 0.85%), and turbidity was visually adjusted to the 0.5 McFarland Standard (1.5×10^8 cells/m*l*). In addition, the absorbance of suspension was determined using a spectrophotometer at 630 nm in a flat-bottom microplate. The suspensions were surface plated within 20 min after preparation, and the following antimicrobial disks were applied to the surface of the plates: rifampin 5 μg (RIF) (BBL), streptomycin (SM) 10 μg (Bioanalyse), ciprofloxacin (CPFX) 5 μg (Oxoid), trimethoprim/sulfamethoxazole (TMP/SMZ) 25 μg (Oxoid), gentamicin (GM) 10 μg (Bioanalyse) and tetracycline (TC) 30 μg (Bioanalyse). On the other hand, vancomycin (VCM) 30 μg (Oxoid), erythromycin (EM) 15 μg (Oxoid), penicillin G (PCG) 10 units (Oxoid), chloramphenicol (CP) 30 μg (Bioanalyse), ampicillin (ABPC) 10 μg (Oxoid), amoxycillin/clavulonic acid (AMPC) 2:1, 30 μg (Oxoid), oxytetracycline (OTC) 30 μg (Oxoid), lincomycin (LCM) 2 μg (Oxoid), enrofloxacin (ERFX) 5 μg (Bioanalyse), polymyxin B (PL-B) 300 units (Oxoid) and cloxacillin (MCIPC) 5 μg (Oxoid) discs were also tested. The plates were incubated in ambient air at 35°C and evaluated after 48 hr. When the inhibition zones around the disks were measured, the results were scored as susceptibility, intermediate and resistant. The *B. melitensis* 16 M reference strain was used as controls for identification, biotyping, PCR and antimicrobial susceptibility testing. In addition, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were also used for susceptibility testing.

The Clinical Laboratory Standards Institute (CLSI; formerly the NCCLS) breakpoints for RIF, CPFX, TMP/SMZ and TC that were for *Heamophilus influenzae* (*H. influenzae*), and GM that was for *Acinetobacter* spp. were accepted for *B. melitensis* in this study [28]. Breakpoints used for SM were recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie [26]. In addition, the following bacteria were considered: *Streptococcus pneumoniae* for VCM, EM and PCG; *H. influenzae* for CP, ABPC and AMPC [28]. For the activity of the other antimicrobials (OTC, LCM, ERFX, PL-B and MCIPC), the interpretations were based on inhibition zone diameter tables for aerobic microorganisms provided by disc suppliers for antibiogram analysis in human and veterinary medicines.

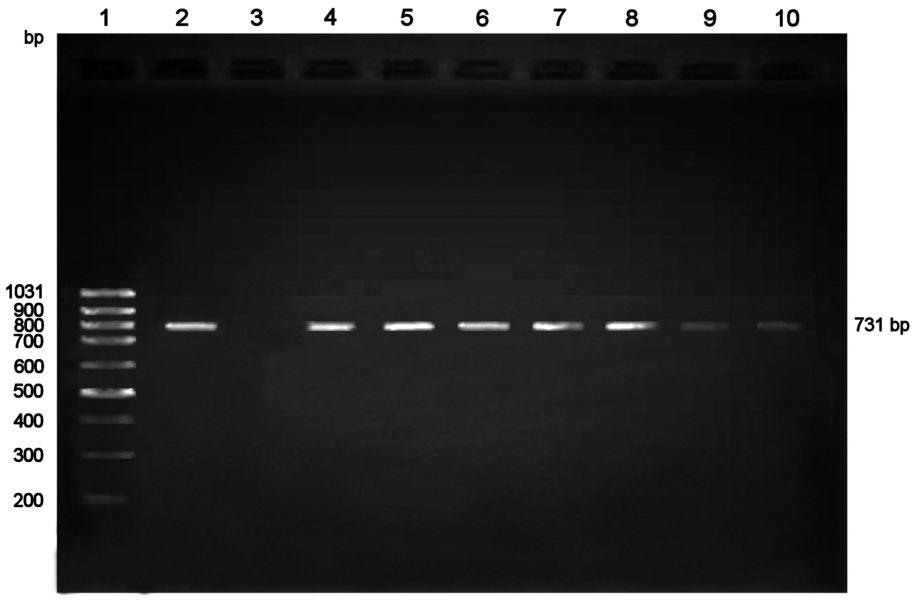

Fig. 1. *Brucella melitensis* PCR products separated on a 1.5% (w/v) agarose gel: line 1, 100 bp ladder DNA molecular size markers (SM0241, Fermentas, Vilnius, Lithuania); line 2, control positive (based on DNA from *B. melitensis* 16 M strain); line 3, control negative (DNases and RNases free water); lines 4-10 PCR positive *B. melitensis* isolates.

RESULTS

All *Brucella* isolates were identified and biotyped as *B. melitensis* biovar 3 by tests, such as positive catalase, oxidase and urease, reduced nitrate, negative H_2S, no CO_2 requirement, no lysis by Tb phage, growth in the presence of thionin and basic fuchsine and positive agglutination with monospecific A and M antisera. Also, all isolates were positive by PCR with *B. melitensis*-species primers (Fig. 1).

The antimicrobial susceptibility test revealed that ovine origin *B. melitensis* strains evaluated in this study were resistant to at least one antimicrobial (51.2%). The resistance was determined to SM, CPFX and GM at the rate of 7.3% and to RIF at the rate of 9.7%. The highest (46.3%) resistance was determined against TMP/SMZ. All isolates were found to be sensitive to TC at the rate of 100.0%. The results of the antibiotic susceptibility testing of 41 *B. melitensis* strains isolated from naturally infected sheep samples are summarised in Table 1.

Table 1. The results of the antibiotic susceptibility testing of 41 *Brucella melitensis* strains isolated from naturally infected sheep samples

Antimicrobial agents	Number of strains (%)		
	Sensitive	Intermediate	Resistant
Rifampin	30 (73.1)	7 (17.1)	4 (9.7)
Streptomycin	38 (92.6)	0 (0.0)	3 (7.3)
Ciprofloxacin	38 (92.6)	0 (0.0)	3 (7.3)
Trimethoprim/ sulfamethoxazole	20 (48.7)	2 (4.8)	19 (46.3)
Gentamicin	38 (92.6)	0 (0.0)	3 (7.3)
Tetracycline	40 (97.5)	1 (2.4)	0 (0.0)
Vancomycin	0 (0.0)	0 (0.0)	41 (100.0)
Erythromycin	29 (70.7)	10 (24.3)	2 (4.8)
Penicillin G	24 (58.5)	10 (24.3)	7 (17.1)
Chloramphenicol	38 (92.6)	0 (0.0)	3 (7.3)
Ampicillin	39 (95.1)	0 (0.0)	2 (4.8)
Amoxycillin/ clavulonic acid	39 (95.1)	0 (0.0)	2 (4.8)
Oxytetracycline	41 (100.0)	0 (0.0)	0 (0.0)
Lincomycin	0 (0.0)	0 (0.0)	41 (100.0)
Enrofloxacin	41 (100.0)	0 (0.0)	0 (0.0)
Polymyxin B	1 (2.4)	19 (46.3)	21 (51.2)
Cloxacillin	0 (0.0)	0 (0.0)	41 (100.0)

DISCUSSION

Since brucellosis is a zoonosis with a strong correlation between animal and human diseases, and the *B. melitensis* is the main causative agent in both human and small ruminant brucellosis [12, 14, 37, 39], in the present study, the focus was on this specific pathogen. According to the findings obtained from the present study, we speculate that an area which is endemic for human brucellosis could have the same particular ecological risk factors for human and favor infection by this agent, since 100% of the isolates from the naturally infected sheep were confirmed as *B. melitensis* biovar 3. The findings of previous reports, in which *B. melitensis* biovar 3 is the most common etiological agent of human in Turkey [2, 7, 9, 10, 34] and animal brucellosis in Turkey [5, 22] and Van region [16, 17] support this speculation.

Therefore, eradication of brucellosis in human populations can only be achieved by the control of the disease in animals as reported by Buzgan *et al.* [12], and this requires a multidisciplinary approach involving both humans and animals.

There are three principal components for the treatment of human brucellosis. Adequate intracellular concentration of antibiotic should be achieved, the antibiotic combination should be chosen because of its synergistic effect and thirdly, *in vitro* susceptibility of the antibiotics should be evaluated [9]. For the treatment of human brucellosis, TC, DOXY, SM, GM, RIF, TMP/SMZ, fluoroquinolones and aminoglycosides have been and are still used [6, 37]. In order to contribute to human clinical medicine, in this study, we investigated the *in vitro* susceptibility of 41 *B. melitensis* strains isolated from naturally infected sheep cases in the region of Van (Eastern Anatolia, Turkey) where human brucellosis is endemic, focusing on RIF, SM, CPFX, TMP/SMZ, GM and TC that are commonly used in human brucellosis. In addition, the other 11 antimicrobials (VCM, EM, PCG, CP, ABPC, AMPC, OTC, LCM, ERFX, PL-B and MCIPC) were also evaluated (Table 1).

Since animal brucellosis is not treated by antibiotics [31, 37], and the susceptibility testing of *Brucella* spp. is not routinely used in human clinical medicine [9], related to the *in vitro* susceptibility, especially against RIF, SM, CPFX, TMP/SMZ, GM and TC have received comparatively attention. Nevertheless, some studies have been performed in different regions of Turkey to assess *in vitro* antimicrobial susceptibility of *Brucella* spp. (especially *B. melitensis*) isolated from humans against different antibiotics. For this purpose, 37 *B. melitensis* isolates from humans were tested against RIF, CPFX, DOXY, TMP/SMZ and ceftriaxone (CFX) in Central Anatolia Region of Turkey. All isolates were determined as sensitive to DOXY, CPFX and CFX, but four (10.8%) isolates to RIF and one (2.7%) isolate to TMP/SMZ were resistant [9]. Altun *et al.* [4] investigated 96 human *Brucella* isolates to RIF, TC, SM, TMP/SMZ, CPFX and tigecycline (TIG) in Central Anatolia. It was concluded that three (3.1%) isolates were found to be resistant to TC, SM and TMP/SMZ. In another study, in the same region of Turkey, 46 *B. melitensis* strains from humans were investigated against RIF, SM, CPFX, TC and azithromycin (AZ). All isolates were sensitive to SM, CPFX, TC and AZ, but two (4.3%) isolates were intermediate sensitivity to RIF [7]. Turan *et al*. [35] reported that 82 *B. melitensis* isolates from humans were analyzed against TIG, DOXY, CPFX, and RIF in Central Anatolia and Mediterranean Regions of Turkey. While all isolates were susceptible to TIG, CPFX and DOXY, intermediate resistance to RIF was detected in 19 (23.1%) isolates. A total of 65 *B. melitensis* isolates from humans were tested to RIF, CPFX, TMP/SMZ, DOXY, ofloxacin (OFX), levofloxacin (LVX) and sparfloxacin (SPX) in the Mediterranean Region. It was stated that eight (12.3%) isolates yielded intermediate resistance to RIF, and the most effective antimicrobial agent was TMP/SMZ, followed by DOXY. With regard to fluoroquinolones, the most active antibiotic was SPX, followed by LVX, CPFX and OFX reported by the same researchers [2]. In the Aegean Region of Turkey, 44 *B. melitensis* strains from blood cultures of humans with acute brucellosis were analyzed against three quinolones [CPFX, LVX and moxifloxacin (MXF)], four macrolides [EM, AZ, dirithromycin (DRM) and clarithromycin (CL)] and DOXY. It was concluded that DOXY was the most active antibiotic and CPFX, and LVX had similar activities. AZ and CL were the most active macrolides followed by EM and DRM [38]. In another study in the same region of Turkey, 34 *B. melitensis* isolates from humans were tested against RIF, SM, CPFX, TC, DOXY, ceftriaxone (CFX) and LVX. It was indicated that five (14.7%) strains had intermediate susceptibility, and one (2.9%) was resistant to RIF [19]. From Saudi Arabia, Kinsara *et al.* [20] reported that of the 37 human *Brucella* isolates, 23 (62.2%) were resistant to TMP/SMZ and all but one (2.7%) isolate sensitive to TC, RIF, GM and CPFX. Memish *et al.* [27] indicated that the *in vitro* resistance rate was 0.6% (one isolate) of 143 human isolates for TC. A total of 41 ovine origin *B. melitensis* isolates were evaluated in this study and determined that the strains were resistant at 51.2% (21 isolates) to at least one antimicrobial. One (2.4%) isolate was resistant to CPFX, 14 (34.1%) isolates to TMP/SMZ, one (2.4%) isolate to TMP/SMZ and GM, three (7.3%) isolates to RIF and TMP/SMZ, one (2.4%) isolate to SM, CPFX and TMP/SMZ and one (2.4%) isolate to RIF, CPFX and TMP/SMZ. A lot of different antibiotics were evaluated in these studies [2, 7, 9, 19, 35, 38], and different results were obtained. These findings support the combinations of the various antibiotics recommendation for the treatment of human brucellosis [6, 37].

Brucella isolates are generally considered susceptible to the antibiotics that recommended by the WHO [37]. Nevertheless, sporadic cases of antibiotic resistance have been reported [9, 23]. In contrast to the other studies [2, 9], in this study, *B. melitensis* isolates were highly resistant (46.3%) against TMP/SMZ, which is consistent with previous reports [4, 19, 25]. There may be a number of reasons why different results were obtained against TMP/SMZ. The disc diffusion method can be affected by many factors, including temperature, pH, type and depth of media, the density of the inoculums used, the interaction between the drug impregnated discs and the agar, incubation time and the addition of calcium, magnesium or sodium salts. In addition, it could be that the *in vitro* susceptibility of antibiotics may change over time and from one geographical region to another [20]. Another explanation for these differences could be that uncontrolled antibiotics used in animals may also cause the development of TMP/SMZ-resistant *B. melitensis* strains. Indeed, some antibiotics have been widely used against some of the infections including abortive diseases in small ruminants in the region of Van. This may indicate that it is necessary to reduce the use of antimicrobials in veterinary medicine. Because of the public health, significance of such resistance may be far larger than the economic losses due to animal brucellosis.

RIF is a potent antibiotic in the treatment of human brucellosis, and it is widely accepted in the best first-line therapy [34]. RIF resistance has been observed *in vitro* in *Brucella* spp. mutants and has shown specific *rpoB* muta-

tions responsible for reduced RIF susceptibility [24, 34]. To our current knowledge, only one RIF-resistant *B. melitensis* strain has been found in human clinical brucellosis cases in Turkey [34]. But, in recent years, *in vitro* activity studies in human isolates in Turkey have shown that the numbers of intermediate susceptible *B. melitensis* isolates to RIF are increasing [2, 9, 19, 34]. For the detection of resistance to RIF, 94 *B. melitensis* isolates from humans in all regions of Turkey (Central Anatolia, Eastern Anatolia, South-Eastern Anatolia, Aegean, Marmara, Black Sea and Mediterranean Regions) were evaluated. It was concluded that all isolates were sensitive to RIF, but two (2.1%) isolates were intermediate susceptible [34]. A total of 56 *B. melitensis* strains isolated from humans with acute brucellosis between 2008–2009 in the region of Van (Eastern Anatolia, Turkey) were tested against RIF, SM, TMP/SMZ and TIG by E-test. It was concluded that the highest MIC values were determined to RIF among the studied antimicrobials [10]. In this study, the resistance to RIF was determined at the rate of 9.7%. This result also supports the results of previous study [10] that carried out in the same area. These findings should be taken into consideration for the potential emergence of RIF-resistant *B. melitensis* infection in this region. So, determination of the regional susceptibility pattern of RIF-resistant *B. melitensis* strains can contribute to further antimicrobial treatment strategies of human brucellosis, and it can contribute to our understanding of their circulation. This led us to think any possible RIF-resistant *B. melitensis* isolates may spread rapidly among regions of Turkey by sacrificial ruminants. But, further works are required to clarify this issue.

It was shown that RIF monotherapy is the main option for brucellosis during pregnancy, whereas its combination with TMP/SMZ is the suggested treatment for childhood brucellosis [14]. In this research, the highest resistance was determined against TMP/SMZ (46.3%) and RIF (9.7%). This may be very important for this endemic region of Turkey due to strategies for the treatment and control of human brucellosis, because people contract the disease by direct contact with contaminated samples or more commonly, as a result of the consumption of contaminated unpasteurized milk and cheese products [12, 37, 39]. In the region of Van, Eastern Anatolia of Turkey, most livestock are owned by smallholders and farmers. Sheep farmers generally produce a local and typical herby cheese (*Otlu Peynir*) preventing standardization of raw milk product, and hygienic conditions are often poor. According to anamnesis, herby cheese consumption was present in 96.5% of the cases in our study area. Therefore, we speculate that sheep and their products, such as herby cheese, may be the most important source of infection and may play a key role in the spread of brucellosis in humans. Therefore, all milk products, especially herby cheese, should be prepared by boiling, or at least acidification process should be applied (should not eat freshly) in the region of Van where human brucellosis is endemic. Briefly this situation, since the resistance to RIF and TMP/SMZ was determined as high in this study, we suggest that periodic assessment of susceptibility of *B. melitensis* isolates to those antibiotics mentioned above should be made to detect any drug resistance as early as possible, so precautions can be taken as quickly as possible in this region.

It was stated that the traditional combination of SM with a TC has been proven to be probably the best currently available therapeutic option for human brucellosis. But, this regime has some disadvantages, such as toxicity. Thus, the use of a quinolone (such as CPFX) in combination with TC or RIF instead of the traditional combination therapy could be considered for the treatment of brucellosis due to the lower toxicity than the TC-SM or TC-RIF combinations [14]. In this study, three *B. melitensis* isolates from ovine were found to be resistant to SM and three isolates to CPFX. So, CPFX may be preferred instead of SM. However, it should be taken into consideration that the results of routine susceptibility tests do not always correlate with clinical efficacy [37].

It was shown that expectations for an ultimate treatment for human brucellosis remain futile, and current experience allows for limited hopes in employing a single antibiotic for a short treatment period with uniform efficacy. New candidates will continue to emerge, although *in vitro* observations do not necessarily translate into *in vivo* facts, thus hampering the overall process [29]. Indeed, in this study, it was determined that ovine origin *B. melitensis* strains were resistant to SM, CPFX and GM at the rate of 7.3%, to RIF at the rate of 9.7% and to TMP/SMZ at the rate of 46.3%.

In conclusion, the results of this study demonstrated that ovine origin *B. melitensis* strains were resistant to at least one of the antimicrobials (51.2%) that have been commonly used in human clinical medicine as basic or alternative antimicrobials. The highest resistance was determined against TMP/SMZ, and a difference was not determined between SM, CPFX and GM.

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