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The canonical *Brucella* species-host dependency is changing, however, the antibiotic susceptibility profiles remain unchanged

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ABSTRACT

Brucellosis is a chronic disease caused by *Brucella* species with a wide range of hosts, from marine mammals to terrestrial species, but with strict host preferences. With the zoonotic character, the prevalence of human brucellosis cases is a reflection of animal infections. This study aimed to identify 192 *Brucella* isolates obtained from various sources by Bruce-ladder PCR and to determine their antibiotic susceptibilities by gradient diffusion method (E-test). As a result of the PCR, all human isolates (n = 57) were identified as *B. melitensis*. While 58 (82.9%) of the cattle isolates were identified as *B. abortus*, 59 (90.8%) of the sheep isolates were identified as *B. melitensis*. In addition, 12 (17.1%) of the cattle isolates and 6 (9.2%) of the sheep isolates were determined as *B. melitensis* and *B. abortus,* respectively. The primary host change behavior of *B. melitensis* was 1.9 times higher than that of *B. abortus*. While gentamicin and ciprofloxacin susceptibilities of *Brucella* isolates were 100%, tetracycline, doxycycline, streptomycin, trimethoprim/sulfamethoxazole and rifampicin susceptibilities were 99%, 99%, 97.4%, 91.7% and 83.9%, respectively. The lowest sensitivity of the isolates was determined against to cefoperazone as 26%. A triple-drug resistance was detected in 1 *B. abortus* isolate that included simultaneous resistance to cefoperazone, rifampicin, and trimethoprim/sulfamethoxazole. The high susceptibility profiles we found against to antibiotics such as tetracycline, doxycycline gentamicin and ciprofloxacin, used widely in treatment, are encouraging. However, the change in the canonical *Brucella* species-primary host preference suggests the need to reconsider eradication program, including updating vaccine formulations.

1. Introduction

Brucellosis, is a zoonotic infection, that causes abortion and infertility in cattle, sheep and goats. The abortion rate due to the *Brucella* species, which have high environmental spread and contagiousness, reaches up to 30–60% in naive herds and causes serious economic losses [1]. Some *Brucella* species can be transmitted to humans through direct contact with infected animals or consumption of infected animal products (especially milk and dairy products) that have not been heat-treated and cause multisystem infection with a broad spectrum of clinical manifestation. The prevalence of the disease is high in the Middle East and Mediterranean countries, Central and South America, Africa and some parts of Asia [2].

The classification of the *Brucella* species is based on biochemical and

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virulence characteristics as well as host preferences. In this respect, 12 *Brucella* species with different characteristics and different host localizations, both human and animal, have been identified currently. *B. melitensis* in sheep and goats, *B. abortus* in cattle and bison, *B. suis* in pigs, hare, reindeer and rodents, and *B. canis* in dogs are the primarily *Brucella* species. These are also the main *Brucella* species responsible for human infections. However, novel *Brucella* species have recently been reported from various human cases [3]. Apart from their primary hosts, *B. melitensis* and *B. abortus* show affinity to different hosts and can cause abortive infections in these hosts [4–7].

The *Brucella* genus includes very close species with high (>98%) genome similarity. Culture-based phenotypical tests (CO_2 requirement, H_2S production, agglutination with monospecific serum and dye sensitivity etc.) are still used in the diagnosis of Brucellosis and in the differentiation of *Brucella* species and biovars [2]. Serological tests (RBPT, SAT, and ELISA) provide more practical results in routine diagnosis. Molecular methods (*Brucella*-genus PCR, AMOS-PCR, Bruce-ladder PCR, RFLP-PCR, VNTR-MLVA, WGS) are widely used in both differential diagnosis and molecular epidemiological studies [8–11].

Treatment of animal brucellosis is not recommended due to its ineffectiveness and inability to completely prevent the carriage of the causative agent [12]. For the treatment of infection in humans, the World Health Organization (WHO) suggests dual or triple combination of antibiotics [1]. However, the intracellular localization and preferred site of the causative agent (e.g. bone marrow) limit the effectiveness of the antibiotics. Tetracyclines (doxycycline or minocycline) combinated with streptomycin and/or rifampicin are the first choice antibiotics for the treatment. Ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole are also used [13]. However, irrational use of antibiotics has revealed high antibiotic resistance among *Brucella* isolates, especially in regions where the infection is endemic such as Egypt, Qatar, Iran, Malaysia and China [2,14]. A comprehensive review also revealed that *Brucellae* are still *in-vitro* susceptible to most of the antibiotics recommended by the WHO, but cases of disease relapse and antibiotic resistance have been documented in North African and Middle Eastern countries, including Turkey [15].

This study aimed to identify *Brucella* species isolated from humans, cattle and sheep in Kars and Kayseri provinces, Türkiye via Bruce-ladder PCR and to analyse the antibiotic susceptibilities of the isolates.

2. Material and methods

2.1. Ethical statement

Ethical approvals of the study were obtained from the Ethics Committee of the Kafkas University Faculty of Medicine (2020/13) and the Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK/2020/103).

2.2. Brucella isolates

In the study, 192 *Brucella* isolates obtained from humans, cattle and sheep in Kars and Kayseri provinces between 2008 and 2020 were used. The isolates were identified by conventional techniques. Human *Brucella* isolates were recovered from blood samples. Cattle and sheep *Brucella* isolates were obtained from vaginal swab samples of aborted animals and aborted fetal tissues.

2.3. Human Brucella isolates

As clinical material, 5–10 ml of whole blood samples taken from patients with suspected brucellosis were used. Isolation of *Brucella* species from blood samples was performed with BACTEC 9120 automated blood culture system (Becton Dickinson, Maryland, USA). Blood samples inoculated into blood culture bottles were incubated for 4 weeks. Samples with positive signals were subcultured on 7% sheep blood agar and incubated at 5% CO_2 for 48 hours. Blind subcultures were carried out once a week. The colonies were identified as *Brucella* spp. considering their macroscopic and microscopic morphologies and Vitek 2.0 Compact system (Biomerieux, Fransa) [16].

2.4. Animal Brucella isolates

The samples taken from tissues such as liver, lung and abomasum content of aborted fetus were directly inoculated into 7% sheep blood agar. The vaginal swab samples belonging to aborted animals were inoculated to Farrell Broth [Brucella Broth Base (BD BBL, 211088), Brucella Selective Supplement (Oxoid SR0083), horse serum (Oxoid SR35) and 1.5% glucose]. Inoculated media were incubated in microaerobic conditions (Anaerocult C, Merck) at 37 °C for 5 days. The pre-enriched samples were then plated on Farrell agar [*Brucella* Medium Base (Oxoid CM0169), horse serum (Oxoid SR35), Brucella Selective Supplement (Oxoid SR0083)] and incubated at 37 °C in a microaerobic environment for 4–7 days [5]. The colonies were identified as *Brucella* spp. considering their macroscopic and microscopic morphologies and various biochemical characteristics [17].

2.5. Molecular identification

2.5.1. Genomic DNA isolation

For DNA extraction, Single cell lysis buffer (SCLB) method was performed from fresh cultures of the *Brucella* isolates [18].

2.5.2. Brucella species-specific PCR (Bruce-ladder PCR)

Species-level identification of the *Brucella* spp. isolates was performed with Bruce-ladder PCR, a multiplex PCR. Vaccine strains and field strains could be distinguished by this method each other [9]. The primers and target genes used in the Bruce-ladder PCR are given in Table 1. Bruce-ladder PCR was consisted of 5 μl 5XLongAmp™ Taq Reaction Buffer, 1 μl $MgCl_2$ (20 mM), 0.75 μl dNTP (10 mM), 1 μl of each primer (12 pieces) (20 pmol), 1 μl LongAmp® Taq DNA Polymerase (5 U) and 3 μl template DNA (50 ng/μl). The thermal condition of Bruce-ladder PCR was set with an initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 35 s, primer binding at 62 °C for 45 s, elongation at 65 °C for 3 min and a final elongation at 65 °C for 10 min. Amplified products were analysed on 1.5% agarose gel. The band sizes obtained were evaluated in the light of the information in Table 1 and *Brucella* isolates were identified at species-level.

2.6. Antibiotic susceptibility test

Antibiotic susceptibility testing was performed and evaluated by gradient diffusion method (E-test) [19]. E-test strips of streptomycin, doxycycline, ciprofloxacin, tetracycline, rifampicin, and gentamicin (Liofilchem, Italy) and trimethoprim/sulfamethoxazole (1:19) and cefoperazone (Himedia, India) were used. After adjusting the density according to McFarland No: 0.5, 100 μl was taken from the 24-48-h fresh cultures of the *Brucella* isolates and inoculated and spread onto Mueller Hinton agar with 5% sheep blood. After 5–10 min drying of the medium, E-test strips were placed and incubated for 48–72 hours at 37 °C under microaerobic conditions (Anaerocult C, Merck). The evaluation was made by determining the points where the inhibition ellipse formed after incubation intersects on the scale on the strip [19].

2.6.1. Evaluation of the antibiotic susceptibility test

Due to Minimum inhibitory concentration (MIC) breakpoints for *Brucella* were not standardized in the Clinical and Laboratory Standards Institute (CLSI) guidelines, the fastidious bacteria (*Haemophilus influenzae)* were used for streptomycin, doxycycline, cefoperazone, ciprofloxacin, tetracycline, rifampicin, gentamicin, and trimethoprim/sulfamethoxazole antibiotics [19]. Resistance to at least one antimicrobial drug in three or more antimicrobial categories observed among

Table 1
Primers and PCR characteristics used for differentiation of the major *Brucella* species [9].

	Brucella species and vaccine strains							Primer	Sequence (5′—3′)	Target gene	Band size (bp)
	Ba	Bm	Bo	Bs	Bc	S19	Rev1				
Gene pattern among the *Brucella* species	◆	◆		◆	◆	◆	◆	BMEI0998f BMEI0997r	ATCCTATTGCCCCGATAAGG GCTTCGCATTTTCACTGTAGC	Glycosyltransferase, *wbo*	1682
		◆	◆	◆	◆		◆	BMEII0843f BMEII0844r	TTTACACAGGCAATCCAGCA GCGTCCAGTTGTTGTTGATG	Outer membrane protein, *omp31*	1071
	◆	◆	◆	◆		◆	◆	BMEI1436f BMEI1435r	ACGCAGACGACCTTCGGTAT TTTATCCATCGCCCTGTCAC	Polysaccharide deacetylase	794
	◆	◆	◆	◆	◆		◆	BMEII0428f BMEII0428r	GCCGCTATTATGTGGACTGG AATGACTTCACGGTCGTTCG	Erythritol catabolism, *eryC*	587
	◆	◆	◆	◆	◆	◆	◆	BMEI0535f BMEI0536r	GCGCATTCTTCGGTTATGAA CGCAGGCGAAAACAGCTATAA	Immunodominant antigen, *bp26*	450
				◆	◆			BR0953f BR0953r	GGAACACTACGCCACCTTGT GATGGAGCAAACGCTGAAG	ABC transporter-binding protein	272
							◆	BMEI0752f BMEI0752r	CAGGCAAACCCTCAGAAGC GATGTGGTAACGCACACCAA	Ribosomal protein S12, *rpsL*	218
	◆	◆	◆	◆	◆	◆	◆	BMEII0987f BMEII0987r	CGCAGACAGTGACCATCAAA GTATTCAGCCCCCGTTACCT	Transcriptional regulator, CRP family	152

Ba: *B. abortus*, Bm: *B. melitensis*, Bo: *B. ovis*, Bs: *B. suis*, Bc: *B. canis*, S19: *B. abortus* S19, Rev1: *B. melitensis* Rev 1.

the *Brucella* isolates was defined as multi-drug resistance (MDR) [20]. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as quality control strains in the study.

3. Results

3.1. Brucella isolates

A total of 192 *Brucella* isolates, 57 human, 70 cattle and 65 sheep isolates from the Kars and Kayseri provinces, were identified. The distribution of the isolates was 99 (51.6%) including 5 human, 41 sheep and 53 bovine *Brucella* isolates from the Kars province, and 93 (48.4%) including 24 sheep, 17 cattle and 52 human *Brucella* isolates from the Kayseri province (Table 2).

3.2. Molecular identification findings

All human isolates were identified as *B. melitensis*. While 58 (82.9%) of the cattle isolates were detected as *B. abortus*, 12 (17.1%) were defined as *B. melitensis*. Fifty-nine (90.8%) and 6 (9.2%) of the sheep isolates were identified as *B. melitensis* and *B. abortus*, respectively. It was determined that all the *Brucella* isolates were identified as field strains (Fig. 1, Table 2). Distributions of *Brucella* species according to the provinces are given in Table 2.

3.3. Brucella species-primary host preference convertion

While the rate of *B. melitensis* isolated from cattle in the Kars province was 20.8%, the rate of *B. abortus* obtained from sheep was 12.2%. The rate of changing the primary host (sheep) preference of *B. melitensis* in the Kars province was 1.7 times higher than *B. abortus* (Odds = 1.7). In the Kayseri province, these rates were calculated as 5.9% and 4.2%, respectively. The rate of changing the primary host (sheep) preference of *B. melitensis* in the Kayseri province was 1.4 times higher than *B. abortus* (Odds = 1.4). When all isolates were considered, while the rate of *B. melitensis* in cattle was 17.1%, the rate of *B. abortus* in sheep was 9.2%. The rate of changing the primary host (sheep) preference of *B. melitensis* was 1.9 times higher than *B. abortus* (Odds = 1.9) (Table 2).

3.4. Antibiotic susceptibility test results

All the *Brucella* isolates were susceptible to gentamicin and ciprofloxacin. Tetracycline, doxycycline, streptomycin, trimethoprim/sulfamethoxazole, and rifampicin susceptibilities of the isolates were determined as 99%, 99%, 97.4%, 91.7%, and 83.9%, respectively (Table 3). Intermediate resistance (I) rate to rifampicin and trimethoprim/sulfamethoxazole were 10.4% and 6.3%, respectively. The susceptibility of the isolates to cefoperazone was 26% (Table 3).

All of the *B. abortus* isolates were susceptible to gentamicin, ciprofloxacin, tetracycline and doxycycline. Susceptibility to streptomycin, rifampicin, trimethoprim/sulfamethoxazole and cefoperazone was found 92.2%, 92.2%, 84.4%, and 15.6%, respectively. Streptomycin resistance was determined only in 5 (8.6%) cattle *B. abortus* isolates and rifampicin resistance in 3 (5.2%) cattle *B. abortus* isolates. Intermediate (I) to rifampicin was detected only in 2 (3.1%) cattle isolates. Fifty-four

Table 2
Species distribution of the *Brucella* isolates according to their hosts.

Location	Origin and *Brucella* spp. (n)	Identification via Bruce-ladder PCR		*Brucella* species-host preference convertion		
		Species	n (%)	Rate (%)	Odds	Convertion status
Kars	Human (5)	*B. melitensis*	5 (100)	ND	ND	ND
	Cattle (53)	*B. melitensis*	11 (20.8)	20.8	1.7	*B. melitensis* - towards cattle
		B. abortus	42 (79.3)	ND		
	Sheep (41)	*B. melitensis*	36 (87.8)	ND		
		B. abortus	5 (12.2)	12.2		
Kayseri	Human (52)	*B. melitensis*	52 (98.1)	ND	ND	ND
	Cattle (17)	*B. melitensis*	1 (5.9)	5.9	1.4	*B. melitensis* - towards cattle
		B. abortus	16 (94.1)	ND		
	Sheep (24)	*B. melitensis*	23 (95.8)	ND		
		B. abortus	1 (4.2)	4.2		
Total	Human (57)	*B. melitensis*	57 (100)	ND	ND	ND
	Cattle (70)	*B. melitensis*	12 (7.1)	17.1	1.9	*B. melitensis* - towards cattle
		B. abortus	58 (82.9)	ND		
	Sheep (65)	*B. melitensis*	59 (90.8)	ND		
		B. abortus	6 (9.2)	9.2		

ND: Not determined.

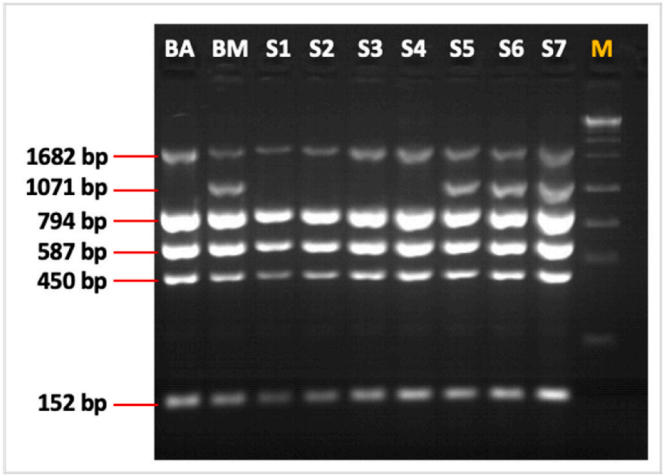

Fig. 1. 1.5% agarose gel electrophoresis images of Bruce-ladder PCR products. BA: *B. abortus* biotype 1 (544), BM: *B. melitensis* biotype 1 (16 M), S1–S4: Field strain (*B. abortus*), S5–S7: Field strain (*B. melitensis*), M: GeneRuler 1 kb DNA Ladder (Thermo Sci SM0311).

(84.4%) *B. abortus* isolates showed cefoperazone resistance. Two (3.1%) of the isolates were resistant to trimethoprim/sulfamethoxazole and 8 (12.5%) were found intermediate (I) (Table 3).

All of the *B. melitensis* isolates were susceptible to streptomycin, ciprofloxacin and gentamicin. Susceptibility to tetracycline, doxycycline, trimethoprim/sulfamethoxazole, rifampicin and cefoperazone were 98.4%, 98.4%, 95.3%, 79.7% and 31.2%, respectively. Tetracycline resistance was found in 1 (1.7%) human and 1 (1.8%) sheep isolates, doxycycline resistance in 1 (8.3%) cattle and 1 (1.7%) sheep isolates, rifampicin resistance in 5 (8.8%) human and 3 (5.8%) sheep isolates and trimethoprim/sulfamethoxazole resistance was detected in 2 (3.4%) sheep isolates. Intermediate (I) to rifampicin was found in 18 (14.1%) isolates. Eighty-eight (68.8%) *B. melitensis* isolates were resistant to cefoperazone, 29 (50.9%) of these were from human, 47 (79.7%) were sheep and 12 (100%) were from cattle origin. Two (1.6%) of the isolates were resistant to trimethoprim/sulfamethoxazole and 4 (3.1%) were intermediate (I) (Table 3).

All human *Brucella* isolates were sensitive to streptomycin, doxycycline, ciprofloxacin, gentamycin and trimethoprim/sulfamethoxazole. The susceptibility to tetracycline, rifampicin and cefoperazone was 98.3%, 75.4%, and 49.1%, respectively. All cattle *Brucella* isolates were susceptible to tetracycline, ciprofloxacin and gentamicin. The susceptibility to doxycycline, streptomycin, rifampicin, trimethoprim/sulfamethoxazole and cefoperazone susceptibilities was 98.6%, 92.9%, 90%, 87.1%, and 12.9%, respectively. All sheep *Brucella* isolates were susceptible to streptomycin, ciprofloxacin and gentamicin. The susceptibility to tetracycline, doxycycline, trimethoprim/sulfamethoxazole, rifampicin and cefoperazone susceptibilities was 98.5%, 98.5%, 89.2%, 84.6%, and 20%, respectively (Table 3).

While tetracycline, doxycycline, ciprofloxacin and gentamicin susceptibilities of the *Brucella* isolates obtained from the Kayseri province were 100%, streptomycin, trimethoprim/sulfamethoxazole, rifampicin, and cefoperazone susceptibilities were 94.6%, 91.4%, 80.7%, and 37.6%, respectively. Although streptomycin, ciprofloxacin and gentamicin susceptibilities of the *Brucella* isolates from the Kars province were 100%, tetracycline, doxycycline, trimethoprim/sulfamethoxazole, rifampicin, and cefoperazone susceptibilities were 99%, 98%, 91.9%, 86.9%, and 15.2%, respectively. The MIC values of all antibiotics used in the study are given in Table 4.

All of the primary host-changing *Brucella* isolates were susceptible to streptomycin, tetracycline, ciprofloxacin, and gentamicin. Two of the isolates were susceptible to rifampicin and one was intermediate (I) to trimethoprim/sulfamethoxazole. Seventeen (94.4%) of the primary host-changing *Brucella* isolates were resistant to cefoperazone. A simultaneous resistance to cefoperazone and doxycycline was detected in one of the primary host-changing isolates (cattle *B. melitensis* isolate

Table 3
Antimicrobial activity test results of the *Brucella* isolates.

Antibiotic	*Brucella* species	Human *Brucella* isolates (%)			Cattle *Brucella* isolates (%)			Sheep *Brucella* isolates (%)			Total *Brucella* isolates (%)		
		S	I	R	S	I	R	S	I	R	S	I	R
STR	*B. melitensis*	57 (100)	-	-	12 (100)	-	-	59 (100)	-	-	128 (100)	-	-
	B. abortus	-	-	-	53 (91.4)	-	5 (8.6)	6 (100)	-	-	59 (92.2)	-	5 (7.8)
TC	*B. melitensis*	56 (98.3)	-	1 (1.8)	12 (100)	-	-	58 (98.3)	-	1 (1.7)	126 (98.4)	-	2 (1.6)
	B. abortus	-	-	-	58 (100)	-	-	6 (100)	-	-	64 (100)	-	-
CFP	*B. melitensis*	28 (49.1)	-	29 (50.9)	-	-	12 (100)	12 (20.3)	-	47 (79.7)	40 (31.3)	-	88 (68.8)
	B. abortus	-	-	-	9 (15.5)	-	49 (84.5)	1 (16.7)	-	5 (83.3)	10 (15.6)	-	54 (84.4)
DOX	*B. melitensis*	57 (100)	-	-	11 (91.7)	-	1 (8.3)	58 (98.3)	-	1 (1.7)	126 (98.4)	-	2 (1.6)
	B. abortus	-	-	-	58 (100)	-	-	6 (100)	-	-	64 (100)	-	-
RD	*B. melitensis*	43 (75.4)	9 (15.8)	5 (8.8)	10 (83.3)	2 (16.7)	-	49 (83.1)	7 (11.9)	3 (5.8)	102 (79.7)	18 (14.1)	8 (6.3)
	B. abortus	-	-	-	53 (91.4)	2 (3.5)	3 (5.2)	6 (100)	-	-	59 (92.2)	2 (3.1)	3 (4.7)
CIP	*B. melitensis*	57 (100)	-	-	12 (100)	-	-	59 (100)	-	-	128 (100)	-	-
	B. abortus	-	-	-	58 (100)	-	-	6 (100)	-	-	64 (100)	-	-
CN	*B. melitensis*	57 (100)	-	-	12 (100)	-	-	59 (100)	-	-	128 (100)	-	-
	B. abortus	-	-	-	58 (100)	-	-	6 (100)	-	-	64 (100)	-	-
TMP/SMZ	*B. melitensis*	57 (100)	-	-	12 (100)	-	-	53 (89.8)	4 (6.8)	2 (3.4)	122 (95.3)	4 (3.1)	2 (1.6)
	B. abortus	-	-	-	49 (84.5)	7 (12.1)	2 (3.5)	5 (83.3)	1 (16.7)	-	54 (84.4)	8 (12.5)	2 (3.1)

S: Sensitive; I: Intermediate; R: Resistant, STR: Streptomycin, TC: Tetracycline, CFP: Cefoperazone, DOX: Doxycycline, RD: Rifampicin, CIP: Ciprofloxacin, CN: Gentamicin, TMP/SMZ: Trimethoprim/sulfamethoxazole.

Table 4
MIC distributions of the *Brucella* isolates (n = 192) according to the CLSI criteria.

Species	Antibiotics	CLSI breakpoints			The number of the *Brucella* isolates with an MIC of:																													
		S≤	I	R≥	0.002	0.003	0.004	0.006	0.008	0.012	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2	3	4	6	8	12	16	24	32	48	64	96	128	192	256	512	1024
B. melitensis	STR	8	-	-													6	24	17	23	25	22	3	1	4			1	1	1												
	TC	2	4	8								4			27	15	21	13	13	15	9	4	4		1					2											
	CFP	1	-	1.5											3		9	8	14		3	2	1		3	4	6	18	57												
	DOX	2	4	8							2	2	11	12	23	13	10	20	16	13			2		2					2											
	RD	1	2	4									3		2	2	12	6	4	1	62	5	5		18				8												
	CIP	1	-	-							1		1		2	2	10	36	61	5	8	1	1																		
	CN	4	-	-												5	12	16	23	15	13	17	13		4	7	3														
	TMP-SMZ	0.5/9.5	1/19-2/38	4/76							1	1	7		11	10	19	9	11	17	9	11	6		3	1	3		3			2	2		1	1					
B. abortus	STR	8	-	-													5	1	9	9	3	13	9	2	2	1	1	2	2	2	3										
	TC	2	4	8								4	2	6	9	9	4	12	3	2	2		4	2	5															
	CFP	1	-	1.5											1			4		2		2	1		1	5	4	10	34												
	DOX	2	4	8							1	3		10	3	11	6	5	3	6	2	7	4		3															
	RD	1	2	4										2	1			4		6	5	5	36		2				3												
	CIP	1	-	-						1	2		2		3			3	3	21	15	4	6	4																	
	CN	4	-	-												1	4	11	10	6	8	4	9	2	1	3	5														
	TMP-SMZ	0.5/9.5	1/19-2/38	4/76							2	1	5	1	3	7	9		4	6	1	3	5				4	2	1		7	1		2							

from Kars province) (Table 5).

Multi-drug resistance (MDR) was detected in 1 (0.52%) *Brucella* isolates. This was a triple-drug resistance detected in 1 *B. abortus* isolate from Kars province, included simultaneous resistance to cefoperazone, rifampicin, and trimethoprim/sulfamethoxazole. Cefoperazone was the antibiotic most frequently included in other MDR candidate combinations (currently resistant to only two antimicrobial compounds tested).

4. Discussion

The classification of *Brucella* species is based on host preferences as well as biochemical and virulence characteristics. In this context, 12 known *Brucella* species are recognized [3]. However, it is not known exactly what the basis of the host preferences of different species is, and in what time period and how they realize this adaptation. It has been suggested that some genetic variations, such as insertions and mutations, may play an important role in the host preferences of the *Brucella* species [21]. The main species of the brucellosis in humans are *B. melitensis, B. abortus, B. suis,* and *B. canis* [22]. Therefore, *Brucella* species causing infection in human are not independent of species in animals, and their incidence largely depends on the prevalence of brucellosis in animals [22,23]. Among these species, *B. melitensis* ranks first in terms of prevalence in human cases [24]. Likewise, in this study, all human isolates were identified as *B. melitensis* as in the other studies [25,26]. *B. melitensis* is the most pathogenic species with its infectivity and high tissue damage and invasion [27]. However, *B. abortus* is less frequent in humans and usually causes subclinical infections [1]. The fact that hospital admissions are more frequent in cases caused by *B. melitensis*, which can lead to overt clinical findings and complaints more easily with these superior characteristics, and that these people are also diagnosed with brucellosis may explain the dominance of *B. melitensis*.

In animals, the canonical *Brucella* species-host relationship is much stronger [27]. Cases where terrestrial *Brucella* species such as *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, and *B. ovis* infect marine mammals, wild animals, or exceptional hosts (vole, baboon, etc.), or vice versa, transmission cases are almost nonexistent [28,29]. However, host specific status changes from time to time among mixed-raised livestock in communal habitats, and cross-infections between host species could occur. Cross-infections caused by *B. abortus* or *B. melitensis* can lead to infertility and abortive infections in sheep and cattle [4–7,30]. In some regions, such as southern Europe and Saudi Arabia, the primary host preference of *B. melitensis* has temporarily changed almost completely [31,32]. The ability of *B. melitensis* to cross-species transmission and establish new reservoirs is also demonstrated by the high identical MLVA genotypes among Turkish isolates obtained from different hosts due to cohabitation [33,34]. Primary host changing has also been reported in bovine abortion cases of *B. melitensis* origin from Kars, Turkey [5,6,35]. In the present study, 20.8% of the bovine *Brucella* isolates from the Kars province were identified as *B. melitensis* and it has been showed that this species continues to play a role as an etiological agent in bovine abortion cases. To the best of our knowledge, this is the first report of

Table 5
Antibiotic susceptibility profiles of the primary host-changing *Brucella* strains.

Code	Location	Host	Species	STR	TC	CFP	DOX	RD	CIP	CN	TMP/SMZ
KY18	Kayseri	Cattle	*B. melitensis*	S	S	R	S	S	S	S	S
KY19	Kayseri	Sheep	*B. abortus*	S	S	S	S	S	S	S	I
KR12	Kars	Cattle	*B. melitensis*	S	S	R	S	S	S	S	S
KR16	Kars	Cattle	*B. melitensis*	S	S	R	S	S	S	S	S
KR17	Kars	Cattle	*B. melitensis*	S	S	R	S	S	S	S	S
KR19	Kars	Cattle	*B. melitensis*	S	S	R	S	S	S	S	S
KR44	Kars	Sheep	*B. abortus*	S	S	R	S	S	S	S	S
KR62	Kars	Sheep	*B. abortus*	S	S	R	S	S	S	S	S
KR63	Kars	Sheep	*B. abortus*	S	S	R	S	S	S	S	S
KR64	Kars	Sheep	*B. abortus*	S	S	R	S	S	S	S	S
KR65	Kars	Sheep	*B. abortus*	S	S	R	S	S	S	S	S
KR67	Kars	Cattle	*B. melitensis*	S	S	R	S	S	S	S	S
KR68	Kars	Cattle	*B. melitensis*	S	S	R	S	S	S	S	S
KR69	Kars	Cattle	*B. melitensis*	S	S	R	S	S	S	S	S
KR70	Kars	Cattle	*B. melitensis*	S	S	R	S	I	S	S	S
KR94	Kars	Cattle	*B. melitensis*	S	S	R	S	S	S	S	S
KR95	Kars	Cattle	*B. melitensis*	S	S	R	R	S	S	S	S
KR96	Kars	Cattle	*B. melitensis*	S	S	R	S	I	S	S	S

B. melitensis with a rate of 5.9% among *Brucella* isolates of bovine origin from the Kayseri province. Reports of *B. abortus* from sheep abortions are quite rare [4,7]. In this study, *B. abortus* positivity among sheep abortion cases was found to be 12.2% for the Kars province and 4.2% for the Kayseri province. Compared to *B. melitensis* (inclination Odds = 1.7), this may be due to the lower potential of *B. abortus* to change its primary host preference and adapt to a new host. Moreover, considering the ability of *B. melitensis* to accumulate genetic mutations more rapidly [36], it may have facilitated the spread of *B. melitensis* to new hosts (spill-over transmission). Cross-infection among hosts by *Brucella* was detected less in the Kayseri province compared to the Kars province. It is thought that factors such as the frequency of the study, the variety and number of samples analysed, the habits of farming different animal species together, rangeland livestock, and modern husbandry practices may affect the primary host changing status.

Treatment of brucellosis in animals is not applied for the inability to completely prevent carriage of the causative agent as well as it is uneconomical and impractical [12]. However, treatment is applied in humans to alleviate the symptoms of the disease and prevent complications and relapses. Treatment protocols for brucellosis vary according to age, pregnancy status, organ involvement, complications, and accessibility and cost of the treatment [37]. The WHO recommends the use of a small number of antimicrobials with proven clinical efficacy and intracellular penetration ability in the treatment of brucellosis in humans. In this context, doxycycline, rifampicin, streptomycin, aminoglycosides and quinolones can be used alone or in appropriate combinations [1,13]. Apart from their use in the initial 2-week period in uncomplicated cases, it is recommended to use aminoglycosides in combination with doxycycline and rifampicin in complications caused by *B. melitensis*, such as endocarditis and spondylitis, and to extend the duration of the treatment [13,37].

A lot of studies dealing with antimicrobial susceptibility testing of *Brucella* species recovered from humans, animals and foods were conducted and these are mainly based the disc diffusion and E-test methods. However, the lack of standardization in the application and interpretation of the tests used, as well as the biological risk involved, make antimicrobial susceptibility detection in *Brucella* species a significant challenge. Nevertheless, continuous susceptibility testing of *Brucella* isolates from different sources, updating of breakpoints and assessing mutations leading to resistance are needed to combat Brucellosis, for which a One Health approach is essential [38].

In humans brucellosis, aminoglycosides such as gentamicin and streptomycin are frequently combined with doxycycline and rifampicin, especially in cases of meningitis, endocarditis and joint and bone involvement [37,39]. In this study, streptomycin susceptibility was defined as 100% in *B. melitensis* isolates, while all *Brucella* isolates were susceptible to gentamicin. This value is consistent with the results of previous studies [38,40–43]. However, the low streptomycin resistance rate (2.6%) we observed for the *B. abortus* isolates was also reported by various researchers [44–46].

Ciprofloxacin can be used in the alternative treatment of brucellosis in humans due to its high bioavailability and intracellular penetration [41]. The higher and lower MIC values of ciprofloxacin were reported for *Brucella* isolates [38,42,47,48]. Similarly to these studies [38,42,48], all *Brucella* isolates recovered from both humans and animals were susceptible to ciprofloxacin with lower MIC values in this study.

Tetracyclines are the most important antibiotics used in the main therapy of human brucellosis and they are suggested by the WHO [49]. Doxycycline has some superior characteristic such as higher intracellular penetration, tissue distribution and increased antimicrobial activity when combined with streptomycin or rifampicin than other tetracyclines [15]. Tetracycline and doxycycline susceptibility, determined as 99% with lower MIC values among the *Brucella* isolates in this study, are consistent with the results obtained in many other studies [40,50,51]. In Turkey, resistance to these two tetracycline group compounds was generally not encountered. With lower rate reported in this study, tetracycline-resistant *B. melitensis* has taken place among the lower to moderate resistance reported in countries such as Iran, Saudi Arabia, Iraq and Syria [15].

Rifampicin is another antibiotic widely used in the treatment of brucellosis in humans due to its high intracellular penetration and synergistic effect gained by drug combinations [52]. On the other hand, the fact that rifampicin breakpoints are not defined in the susceptibility tests of the *Brucella* isolates and the values of slow-growing model bacteria are used instead sometimes causes confusion in the interpretation of test results [53]. In our study, 83.9% of the *Brucella* isolates were susceptible to rifampicin and 10.4% were intermediate, and these rates obtained are compatible with the other studies [41,54,55]. However, it is noteworthy that rifampicin resistance was 8.8% and 4.4% among the human and animal *Brucella* isolates, respectively. Together with other studies conducted in Turkey [54,56,57], it can be said that the rifampicin resistance of the *Brucella* isolates is lower than 10%. However, this resistance to rifampicin should be taken into consideration since it is also used in the treatment of *Mycobacterium tuberculosis* in many developing countries including Turkey [58].

Trimethoprim-sulfamethoxazole combined with rifampicin and aminoglycosides is used in the treatment of brucellosis in children and pregnant women [49]. However, different opinions have also been reported regarding susceptibility tests and their efficacy in the treatment. Both high rates of susceptibility [25,54] and various resistance rates (2.4%–23.2%) have been reported in *Brucella* isolates against this antibiotic [55,56,59]. In this study, trimethoprim-sulfamethoxazole susceptibility of the *Brucella* isolates was 91.7% with a broad MIC range. Due to its use in the treatment of special groups in humans, the number of studies mentioning trimethoprim-sulfamethoxazole resistance is higher when compared to the animals' reports and has been reported in recent years, especially in Iran [15,43]. In this study, in contrast, trimethoprim-sulfamethoxazole resistance was not found among the *Brucella* isolates in humans. The resistant *Brucella* isolates to this antimicrobial were from cattle and sheep origin only. This may be due to the widespread use of trimethoprim in the treatment of various infections in animals, at least as much as used in humans.

Third-generation cephalosporins are widely used in the treatment of neurobrucellosis due to their high penetration into the cerebrospinal fluid [60]. Ceftriaxone is the most common antibiotic whose antimicrobial activity has been tested [25,43,61]. However, studies on the use of cefoperazone, a 3rd generation cephalosporin, are limited [62]. In this study, cefoperazone resistance was found in 74% of the *Brucella* isolates, while the resistance was higher (84.4%) among the *B. abortus* isolates. In addition, cefoperazone resistance rate was found as 94.4% among *Brucella* isolates that changed the primary host. The MIC range of cefoperazone was not very wide for the susceptible isolates. However, in the case of resistance to ceftriaxone in the treatment of neurobrucellosis, it can be considered as a better-than-nothing compound.

Multi-drug resistance (MDR) has been reported among *Brucella* species obtained from different sources and locations. This resistance is most frequently caused by ciprofloxacin, streptomycin, trimethoprim/sulfamethoxazole and rifampicin, and the rate has been determined between 1.4% and 96% [2,48,63]. In this study, a triple-drug resistancewas found 0.5% among the *Brucella* isolates. Antibiotics responsible for the MDR were cefoperazone, rifampicin and trimethoprim/sulfamethoxazole in this study. The MDR positivity among the *Brucella* isolates was lower as reported in other studies [2,48,63]. It was also noteworthy that the MDR positivity was detected in one cattle *B. abortus* isolate. This may be due to the lack of specific, low-dose, and definite-term use of antibiotics in animals compared to humans.

5. Conclusion

In summary, the high susceptibility rates we found against antibiotics such as gentamicin, ciprofloxacin, tetracycline, and doxycycline, which are frequently used in the treatment of brucellosis and are easily

accessible, are encouraging. However, the change in the canonical *Brucella* species-host preference may lead various problems. Reorganizing eradication practices, updating vaccine formulations, and developing polyvalent vaccines are thought to be necessary to solve these problems. In addition, it should be considered that *B. melitensis* can also be transmitted to humans from cattle and treatment regimens should be planned accordingly. The present study, which carried out to reveal *Brucella* species diversity in both human and animal infections and to determine the antibiotic susceptibilities of the species, will strengthen the agent-specific control.

CRediT authorship contribution statement

Elif Celik: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tuba Kayman:** Methodology, Formal analysis, Data curation, Conceptualization. **Fatih Buyuk:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aliye Gulmez Saglam:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Secil Abay:** Writing – review & editing, Validation, Supervision. **Mustafa Akar:** Writing – review & editing, Validation, Supervision. **Emre Karakaya:** Validation, Resources, Methodology. **Cigdem Eda Balkan Bozlak:** Resources, Formal analysis, Conceptualization. **Mustafa Reha Coskun:** Formal analysis, Data curation, Conceptualization. **Eray Buyuk:** Data curation, Conceptualization. **Ozgur Celebi:** Methodology, Formal analysis, Conceptualization. **Mitat Sahin:** Writing – review & editing, Writing – original draft, Validation. **Izzet Burcin Saticioglu:** Writing – review & editing, Writing – original draft, Validation. **Seda Durhan:** Resources, Investigation, Formal analysis. **Atakan Baykal:** Investigation, Formal analysis, Conceptualization. **Yaren Ersoy:** Writing – original draft, Methodology, Investigation, Formal analysis. **Salih Otlu:** Writing – review & editing, Writing – original draft, Validation. **Fuat Aydin:** Writing – review & editing, Writing – original draft, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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