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BMC Infectious Diseases (2024) 24:1220
https://doi.org/10.1186/s12879-024-09921-w

BMC Infectious Diseases

RESEARCH

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Whole-genome sequencing-based analysis of *Brucella* species isolated from ruminants in various regions of Türkiye

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Abstract

Background Brucellosis, a zoonotic disease in Türkiye, which has significant direct and indirect impacts on the healthcare system and livestock. This study, which aimed to investigate the differences among *Brucella* spp. isolates originating from different regions of Türkiye, for implications for public health and veterinary medicine.

Method Twenty-one isolates from ruminants and two isolates from humans obtained from various regions of Türkiye were utilized in the study. The isolates were identified and biotyped using traditional microbiological procedures, and whole-genome sequencing (WGS) was performed. This was followed by single nucleotide polymorphism (SNP)--based phylogenetic analysis and WGS-based analysis of virulence and resistance genes. Additionally, phenotypic antimicrobial resistance and phage susceptibilities were determined. The obtained data were then compared for concordance, ensuring the validity and reliability of the results.

Results Our study, employing culture methods, polymerase chain reaction (PCR), and WGS analyses, identified 11 *Brucella melitensis* (bv 3 ($n=9$), one each bv 1 and bv 2) and 12 *B. abortus* (bv 3 ($n=11$), bv 9 ($n=1$)) isolates All *B. abortus* isolates were of bovine origin, while the *B. melitensis* isolates were from sheep ($n=7$), goat ($n=1$), ram ($n=1$), and humans ($n=2$). In the whole-genome SNP-based phylogenetic tree, all *B. melitensis* strains were found to be of the IIb subtype of genotype II associated with the Eastern Mediterranean lineage. Ten different genotypes were identified in the SNP analysis of the isolates, with a maximum SNP difference of 278 and a minimum SNP difference of 4 among these genotypes. According to the WGS-SNP-based phylogenetic tree of *B. abortus* isolates, they were grouped in clade C1. In the SNP analysis, where ten different genotypes were identified, the SNP difference among these genotypes was a maximum of 316 and a minimum of 6.

In the in silico MLST analysis performed with WGS data, *B. melitensis* isolates were identified as ST8 and ST102 genotypes, while *B. abortus* isolates were identified as ST2 and ST3 genotypes. The dominant genotypes were ST8 for *B. melitensis* and ST2 for *B. abortus*, respectively. Virulence gene analysis conducted based on WGS data of the 23 *B. abortus* and *B. melitensis* isolates revealed 43 virulence gene-associated regions in all strains, irrespective of species, host, or isolation year. Although classical resistance-related genes were not detected by WGS-based antimicrobial resistance gene analysis, phenotypic resistance analysis revealed resistance to azithromycin, rifampin, and trimethoprim/sulfamethoxazole in *B. abortus* and *B. melitensis* isolates.

Conclusion Both *B. melitensis* and *B. abortus* were circulating species in animals and human. The dominant genotypes were ST8 for *B. melitensis* and ST2 for *B. abortus*, respectively. All *B. melitensis* strains were found to be of the IIb

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subtype of genotype II associated with the Eastern Mediterranean lineage, while *B. abortus* isolates, they were grouped in clade C1. Further, a comprehensive study with a sufficient number of isolates covering all regions of Türkiye would provide more accurate information about the current epidemiological situation in the country.

Keywords *B. abortus*, *B. melitensis*, MLST, Ruminants, Türkiye, WGS

Introduction

Brucellosis has been known for many years to affect both animal and human health. According to the World Health Organization [1–4], brucellosis is one of the most neglected common zoonoses with estimated more than 2 million cases of brucellosis being reported annually worldwide [5]. Although brucellosis is a notifiable disease in many countries, its incidence is much greater than reported, partly due to the nonspecific nature of clinical symptoms. As infected humans do not pose a significant risk to their surroundings and human-to-human transmission rarely occurs, monitoring of natural animal reservoirs is crucial for reducing the incidence of brucellosis [1, 4–7]. In Türkiye, the disease is commonly observed in both humans and animals. A prerequisite for controlling the disease is to implement a One Health approach, conducting multidisciplinary studies to control the disease in both farm and wildlife populations [2, 8–10].

B. melitensis, *B. abortus*, and *B. suis* are the main causative agents of brucellosis [11]. These can lead to serious infections in both animals and humans and often result in significant economic losses [11, 12]. *B. abortus* is the main species associated with bovine brucellosis and consists of seven different biotypes (bv), including 1, 2, 3, 4, 5, 6, and 9. The main agent that causes sheep-goat brucellosis is *B. melitensis*, which has three biotypes: bv 1, 2, and 3. *B. suis* is known for its ability to infect swine; B. *suis* is divided into five biotypes: bv 1, 2, 3, 4, and 5 [11, 13, 14]. Brucellosis is endemic (and hyperendemic in some regions) in Türkiye [8, 9]. The disease caused by *Brucella* species and biotypes is a significant financial burden on animal and public health in developing countries such as Türkiye [1, 6] and can only be overcome with effective diagnosis and epidemiological assessment of dominant *Brucella* species.

Classic bacteriological, serological, and molecular tests assist in disease diagnosis but are insufficient for analyzing the relationships and evolution among circulating genotypes [15, 16]. Studies based on multiple-locus variable number tandem repeat analysis (MLVA), multilocus sequence typing (MLST), and increasingly dominant techniques, such as whole-genome sequencing (WGS), have revealed the genetic relationships, geographic origins, and the genotypes that exist within species [15, 17, 18]. Such studies enable the geographical clustering of isolates, the tracking of sources of infection, and help determine global distribution. Unlike MLVA and MLST, WGS relates to the entire genome rather than to specific regions. Thus, the characterization of all existing variations in the genome allows for more sensitive evaluations [15, 18]. WGS is increasingly being used in various fields, standing out as a versatile technique for determining pathogen evolution and phylogenetic relationships, as well as identifying resistance and virulence genes [19]. With the development of next-generation sequencing methods, the routine use of WGS has become possible [19, 20]. With advancements in bioinformatics, the use of WGS has increased and has played a significant role in uncovering the dynamics of many infections today [21–24].

This study aimed to perform a whole-genome sequencing analysis of *Brucella* species originating from various regions of Türkiye. Within this scope, WGS-single nucleotide polymorphism (SNP)--based phylogenetic analysis, WGS-based virulence gene, and resistance gene analysis were performed. Besides, identification and biotyping methods, phenotypic antimicrobial resistance, and phage susceptibility analyses were conducted on the isolates.

Materials and methods

Origin of *Brucella* isolates

A total of 23 *Brucella* isolates obtained from cattle ($n=12$), sheep ($n=7$), humans ($n=2$), one ram ($n=1$), and one goat ($n=1$) were used in the study. These strains were isolated from blood, aborted fetuses, milk, vaginal, and preputial swab samples taken from small and large ruminants, as well as from humans for diagnostic purposes, from various regions of Türkiye (Table 1).

Brucella isolates and identification

Before biotyping procedures began, all of the isolates were tested for catalase, oxidase, and urease activity. The phage sensitivity of the isolates was evaluated using Izatnagar, Tbilisi (RTD and 10^4 RTD), Berkeley, Weybridge, and R/C phages. *Brucella* isolates were biotype based on CO_2 requirement, H_2S production, sensitivity to dyes basic fuchsin (Sigma) (20 µg/mL), thionine (Sigma) (20 µg/mL), Safranin O (Sigma) (100 µg/mL), and agglutination with A and M monospecific sera (2010-1; PVKE). To differentiate from vaccine strains, all of the isolates were grown in a medium containing streptomycin

Table 1 Origin information of the isolates

İsolate No	Region (City)	Host	Sample	Year
22RB24664 (1)	Iğdır	Cattle	Milk	2022
22RB24665 (2)	Iğdır	Cattle	Milk	2022
22RB24666 (3)	Şanlıurfa/Harran	Ram	Preputial swab	2021
22RB24667 (4)	Iğdır	Cattle	Vaginal swab	2022
22RB24668 (5)	Bingöl	Human	Blood	2021
22RB24669 (6)	Bingöl	Human	Blood	2021
22RB24670 (7)	İstanbul	Sheep	Fetus	2017
22RB24671 (8)	İstanbul	Sheep	Fetus	2017
22RB24672 (9)	Şanlıurfa/Akçakale	Cattle	Milk	2022
22RB24673 (10)	Şanlıurfa/Haliliye	Sheep	Fetus	2022
22RB24674 (11)	İstanbul	Cattle	Fetus	2017
22RB24675 (12)	Diyarbakır	Sheep	Fetus	2021
22RB24676b (13)	Diyarbakır	Cattle	Fetus	2021
22RB24677 (14)	Diyarbakır	Cattle	Fetus	2021
22RB24678 (15)	İstanbul	Cattle	Vaginal swab	2017
22RB24679 (16)	Şanlıurfa/Hilvan	Cattle	Milk	2022
22RB24681 (18)	Iğdır	Cattle	Milk	2022
22RB24682 (19)	Bursa	Cattle	Milk	2022
22RB24683 (20)	Bursa	Cattle	Milk	2022
22RB24684b (21)	Elazığ	Sheep	Vaginal swab	2022
22RB24685 (22)	Siirt	Goat	Vaginal swab	2021
22RB24686 (23)	Elazığ	Sheep	Vaginal swab	2022
22RB24687b (24)	Elazığ	Sheep	Vaginal swab	2022

(Sigma) (2.5 µg/ mL), penicillin (Merck) (5 IU/mL), and i-erythritol (Sigma) (1 mg/mL), as previously described [11, 25]. Genomic DNA was extracted using a commercial kit (Invitrogen), while isolates were confirmed using multiplex PCR (Bruce-Ladder) [26, 27]. Phenotypic antimicrobial resistance analysis was conducted using the disk diffusion method reported for *Haemophilus influenzae* [28, 29]. Antibiotic disks used (Bioanalyse) in the antibiogram included doxycycline 30 µg (DO), azithromycin 15 µg (AZM), gentamicin 10 mcg (CN), rifampin 5 mcg (RA), and trimethoprim/sulfamethoxazole 25 mcg (TS).

Whole-genome sequencing of *Brucella* isolates

Whole-genome sequencing was conducted according to the methods previously applied by Brangsch et al. [30]. Sequencing libraries were prepared using the Nextera XT library preparation kit (Illumina, San Diego, CA, USA) protocol. Paired-end sequencing with 2×300 bp read lengths was performed on a MiSeq system using v3 chemistry (Illumina Inc., San Diego, CA, USA). FastQC v0.11.7 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to assess the quality metrics of the Illumina sequence data, while Kraken2 v2.0.7_beta was utilized for contamination control and species identification validation. Genome assembly was conducted using Shovill (v. 1.0.4) (//github.com/tseemann/shovill) for read trimming with trimmomatic and merging paired-end read. Assembly was then performed using SPAdes, following a similar approach to Shovill. A quality assessment of the de novo assembled contigs was conducted using QUAST v.5.0.2 and Prokka v.1.14.5.

SNP calling of the genome was conducted using Snippy v 4.6.0 (https://github.com/tseemann/snippy), with *B. abortus* 2308 and *B. melitensis* 16 M being used as reference genomes. A core genome alignment was generated from identified SNPs using Snippy, and the number of different SNPs was calculated using snp-distsv0.7.0 (https://github.com/tseemann/snp-dists). Genome SNP alignments served as the basis for a maximum likelihood analysis conducted using RAxML (randomized axial maximum likelihood) (v 8.2.12). The resulting tree was visualized using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). For SNP analysis, raw read data for 32 *B. melitensis* and 21 *B. abortus* strains available in the NCBI Sequence Read Archive were browsed (data was checked against methods used for local isolates). Based on the assemblies, in silico multilocus sequence typing (MLST-9) was conducted via mlst v 2.19.0 (https://github.com/tseemann/mlst).

Antimicrobial resistance genes were detected using the Comprehensive Antibiotic Resistance Database (CARD) [31]. In silico investigation of the *mecA*, *tetA*, *tetG*, *folA*, *ermA*, *ermB*, *ermC*, *mefA*, and *msrA* genes was carried out using the assembled genomes of the isolates. Virulence-associated genes were analyzed using the Virulence Factor Database (VFDB, http://www.mgc.ac.cn/VFs/) based on the assembled genomes of the *Brucella* species included in this thesis project [32].

Results

According to findings from conventional identification, isolates from large ruminants were identified as *B. abortus*, whereas those isolated from small ruminants and humans were identified as *B. melitensis*. Based on biotyping of 12 *B. abortus* isolates, 11 were typed as *B. abortus* bv 3, while one isolate originating from Diyarbakır (22RB24676b) was typed as *B. abortus* bv 9 (Table 2). Evaluation of *B. melitensis* isolates, based on biotyping, revealed that out of 11 isolates, 9 were typed as *B. melitensis* bv 3, with one isolate being typed as *B. melitensis* bv 1, and one as *B. melitensis* bv 2. While four *B. melitensis* bv 3 isolates from antibiotic-containing and dye media were found to be penicillin-sensitive, one isolate from sheep in Diyarbakır (22RB24675) was typed safranin-sensitive *B. melitensis* bv 1. Interestingly, the ram isolate, which originated from Şanlıurfa

Table 2 Identification of *B. melitensis* isolates at species and biotype level by culture method

Growth in Stained Media					Growth in Antibiotic Medium					Lysis Test with Phages			Anti Monospecific Serum Test		
Isolate No	Host	Basic fucsin	Thionine	Safranin	Penicillin	Eryhtritol	Streptomycin	CO_2 needs	H_2S production	Izatnagar	Tbilisi	R/C	Anti-A	Anti- M	Biovar
22RB24666	Ram	+	+	-	-	+	+	-	-	+	-	-	+	-	Streptomycin resistant, safranin and penicillin sensitive *B. melitensis* bv-2
22RB24668	Human	+	+	+	-	+	-	-	-	+	-	-	+	+	Penicillin sensitive *B. melitensis* bv-3
22RB24669	Human	+	+	+	-	+	-	-	-	+	-	-	+	+	Penicillin sensitive *B. melitensis* bv-3
22RB24670	Sheep	+	+	+	+	+	-	-	-	+	-	-	+	+	*B. melitensis* bv-3
22RB24671	Sheep	+	+	+	+	+	-	-	-	+	-	-	+	+	*B. melitensis* bv-3
22RB24673	Sheep	+	+	+	-	+	-	-	-	+	-	-	+	+	Penicillin sensitive *B. melitensis* bv-3
22RB24675	Sheep	+	+	-	+	+	-	-	-	+	-	-	-	+	Safranin sensitive *B. melitensis* bv-1
22RB24684b	Sheep	+	+	+	+	+	-	-	-	+	-	-	+	+	*B. melitensis* bv-3
22RB24685	Goat	+	+	+	-	+	-	-	-	+	-	-	+	+	Penicillin sensitive *B. melitensis* bv-3
22RB24686	Sheep	+	+	+	+	+	-	-	-	+	-	-	+	+	*B. melitensis* bv-3
22RB24687b	Sheep	+	+	+	+	+	-	-	-	+	-	-	+	+	*B. melitensis* bv-3

Table 3 Identification of *B. abortus* isolates at species and biotype level by culture method

		Growth in Stained Media			Growth in Antibiotic Medium					Lysis Test with Phages			Anti Monospecific Serum Test		
Isolate No	Host	Basic fucsin	Thionine	Safranin	Penicillin	Eryhtritol	Streptomycin	CO_2 needs	H_2S production	Izatnagar	Tbilisi	R/C	Anti-A	Anti- M	Biovar
22RB24664	Cattle	+	+	+	+	+	-	+	+	+	+	-	+	-	*B. abortus* bv-3
22RB24665	Cattle	+	+	+	+	+	-	+	+	+	+	-	+	-	*B. abortus* bv-3
22RB24667	Cattle	+	+	+	+	+	-	+	+	+	+	-	+	-	*B. abortus* bv-3
22RB24672	Cattle	+	+	+	+	+	-	+	+	+	+	-	+	-	*B. abortus* bv-3
22RB24674	Cattle	+	+	+	+	+	-	+	+	+	+	-	+	-	*B. abortus* bv-3
22RB24676b	Cattle	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. abortus* bv-9
22RB24677	Cattle	+	+	+	+	+	-	+	+	+	+	-	+	-	*B. abortus* bv-3
22RB24678	Cattle	+	+	+	+	+	-	+	+	+	+	-	+	-	*B. abortus* bv-3
22RB24679	Cattle	+	+	+	-	+	-	+	+	+	+	-	+	-	Penicillin sensitive *B. abortus* bv-3
22RB24681	Cattle	+	+	+	+	+	-	+	+	+	+	-	+	-	*B. abortus* bv-3
22RB24682	Cattle	+	+	+	+	+	-	+	+	+	+	-	+	-	*B. abortus* bv-3
22RB24683	Cattle	+	+	+	+	+	-	+	+	+	+	-	+	-	*B. abortus* bv-3

(22RB24666), was found to be resistant to streptomycin but sensitive to penicillin and safranin (Table 3).

PCR confirmed the identification of 12 isolates originating from cattle as *B. abortus*. The other 11 isolates from small ruminants and humans were confirmed as being *B. melitensis* (Fig. 1).

Regarding phage susceptibility with Berkeley, Izatnagar, R/C, Tbilisi, and Weybridge phages, all 11 *B. melitensis* isolates were lysed by Izatnagar Φ, while no lysis was observed with R/C and Tbilisi phages. Seven *B. melitensis* isolates were lysed with Berkeley Φ, while two isolates formed phage plaques with Weybridge Φ, and these isolates also formed phage plaques with Berkeley Φ. All 12 *B. abortus* isolates formed full lysis with the tested phages, except for R/C Φ, which only lyses rough strains.

According to an antibiogram test, which was performed using the disk diffusion method with five antimicrobial agents (DO, CN, AZM, RA, TS), no resistance to doxycycline and gentamicin was detected in the *B. melitensis* ($n=11$) and *B. abortus* ($n=12$) isolates (100% sensitivity observed). High resistance to azithromycin was observed, with 100% of *B. melitensis* isolates and 91.66% of *B. abortus* isolates being resistant to this antibiotic. A total of 45.45% of the *B. melitensis* isolates and 58.3% of the *B. abortus* isolates were resistant to rifampin. The resistance rates of the *B. melitensis* and *B. abortus* isolates to trimethoprim/sulfamethoxazole were 18.18% ($n=2$) and 25% ($n=3$), respectively (Table 4). Three *B. abortus* isolates resistant to trimethoprim/sulfamethoxazole were also found to be resistant to rifampin, while two *B. melitensis* isolates resistant to trimethoprim/sulfamethoxazole were found to be sensitive to rifampin.

Genome sequencing analysis

Twenty-three *Brucella* spp. isolates were sequenced. According to the FastQC analysis, there were no problems with the sequence qualities. The read counts for the isolates ranged between a minimum of 2,651,264 and a maximum of 11,758,676. The GC content of all isolates was determined as being 57% (Table 5).

The genomic diversity of the isolates was evaluated using in silico MLST analysis of genome sequencing data. All of the 11 *B. melitensis* isolates belonged to the Eastern Mediterranean lineage. Eight isolates were classified as having the sequence type (ST) 8 genotype, and 3 (22RB24670, 22RB24671, 22RB2467) as having the ST102 genotype. Among the *B. abortus* isolates, 10 (83.33%) were genotyped as ST2. There were also two *B. abortus* isolates from Iğdır Province, which originated from bovine milk samples (22RB24664 and 22RB24665). These were identified as having the ST3 genotype (Table 5).

The WGS data of 11 *B. melitensis* isolates originating from various regions of Türkiye were compared with the genome data of 32 *B. melitensis* strains available in public databases. A WGS-SNP-based phylogenetic tree was constructed with these strains assigned to Genotypes I, II, III, IV, and V. It was determined that all local isolates were associated with the Eastern Mediterranean lineage within Genotype II (Fig. 2). The local isolates ($n=11$) clustered within the IIb subgenotype of the Eastern Mediterranean lineage, showing high genetic similarity. In the SNP analysis of the eleven isolates, ten different genotypes were identified, with a maximum SNP difference of 278 and a minimum SNP difference of 4 among these genotypes. The local isolates were observed to divide into three groups: Diyarbakır (22RB24675) and Istanbul (22RB24670, 22RB24671) isolates formed one group, while the Elazığ isolates (22RB2484b, 22RB2486, 22RB2487b), isolated from the same nomadic sheep flock, formed another group with a maximum difference of 4 SNPs among them. The Siirt (22RB2485), Bingöl (22RB24668, 22RB24669), Şanlıurfa/Harran (22RB24666), and Şanlıurfa/Haliliye (22RB24673) isolates constituted the third group. No significant differences were observed between the human (22RB24668, 22RB24669) and animal isolates (Table 6). For *B. abortus* strains, represented by the A, B, and C (C1, C2) clades, the genomic data of 21 *B. abortus* strains obtained from public data sources and 12 *B. abortus* isolates from various geographical regions of Türkiye were used to construct a WGS-SNP-based phylogenetic tree. All Turkish isolates were clustered within the C1 clade, similar to strains originating from Iran, China, and Iraq (Fig. 3). In the SNP analysis, where ten different genotypes were identified, the SNP difference among these genotypes ranged from a maximum of 316 to a minimum of 6. Among the local B. abortus isolates in clade C1, which were divided into two main groups, the isolates from Iğdır ($n=4$), along with the Şanlıurfa/Hilvan (22RB24679) and Istanbul (22RB24674) isolates, clustered in the region where the Iranian-origin strains were also located, while the remaining local isolates ($n=6$) clustered in the region where the Chinese-origin strain was located (Table 7).

In silico antimicrobial resistance gene analysis was conducted using the Comprehensive Antibiotic Resistance Database (CARD), but no gene associated with antibiotic resistance was detected in the genomes of any of the local *Brucella* isolates included in the study.

Virulence gene analysis was performed using VFDB based on the whole-genome sequence data of 11 *B. melitensis* and *12 B. abortus isolates* included in the study. The analysis identified 43 virulence-associated gene regions (*acpXL*, *fabZ*, *gmd*, *htrB*, *kdsA*, *kdsB*, *lpsA*,

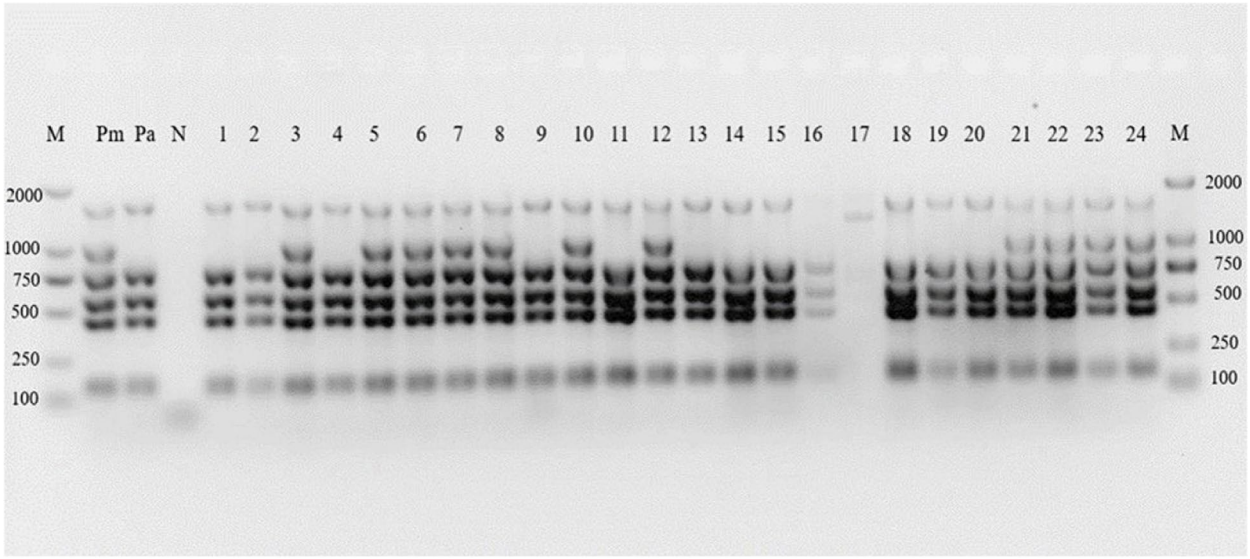

Fig. 1 Gel electrophoresis image of samples following PCR. M: Ladder, Pm: Positive control *B. melitensis*, Pa: Positive control *B. abortus*, N: Negative control, 1,4,9,11,13–16,18–20: *B. abortus* field isolates, 3,5–8,10,12,21–24: *B. melitensis* field isolates

Table 4 Antimicrobial resistance rates of *Brucella* isolates

Antibiotics	S	I	R	*B. abortus*			*B. melitensis*		
				S %/n	I %/n	R %/n	S %/n	I %/n	R %/n
Azithromycin (15 µg)	≥12	-	-	9.33/1	0	91.66/11	0	0	100/11
Doxycycline (30 µg)	10≥	-	-	100/12	0	0	100/11	0	0
Gentamicin (10 mcg)	≥16	-	-	100/12	0	0	100/11	0	0
Rifampin (5 mcg)	≥20	17-19	≤16	41.66/5	0	58.33/7	54.54/6	0	45.45/5
Trimethoprim Sulfamethoxazole (25 mcg)	≥16	11-15	≤10	50/6	25/3	25/3	81.81/9	0	18.18/2

lpsB/lpcC, *lpxA*, *lpxB*, *lpxC*, *lpxD*, *IpxE*, *manAoAg*, *manCoAg*, *wbdA*, *wbkA*, *wbkB*, *wbkC*, *wboA*, *wbpL*, *wbpZ*, *wzt*, *wmz*, *per*, *pgm*, *pmm*, *virB10*, *virB11*, *virB12*, *virB1*, *virB2*, *virB3*, *virB4*, *virB5*, *virB6*, *virB7*, *virB8*, *virB9*, *btpA*, *btpB*, *RicA*, *and Cgs*).

Discussion

Brucellosis continues to be a significant zoonotic disease that affects many parts of the world [1, 33, 34]. Particularly in developing countries, the lack of multidisciplinary collaboration among stakeholders such as farmers, animal and human health authorities, and political leaders is a major factor hindering disease control and prevention strategies [35]. The causative agents of the disease, *Brucella* spp., are defined based on host preference, as well as phenotypic and genotypic characteristics [11, 36]. Advances in diagnostic methods have expanded the genus *Brucella* by adding new members and allowing for more detailed species analysis [16]. Techniques such as MLVA, MLSA, and increasingly dominant WGS-based analyses all reveal the genetic relationships, geographic origins, and genotypes that exist within species [16, 36, 37]. These techniques enable geographical clustering of *Brucella* spp. isolates, tracking of sources of infection, and the determination of global distribution [16, 36–38]. With WGS, all variations present in the genome can be finely characterized, making studies using these approaches ideal for a better understanding of pathogens today [36–38].

In the present study, WGS-SNP analysis and virulence and resistance gene analyses were conducted using the complete genome sequences of 21 *Brucella* isolates from different geographical regions of Türkiye, as well as two human isolates. The isolates were also examined using classical culture identification and biotyping, multiplex PCR, phenotypic antimicrobial resistance, and phage sensitivity analyses. SNP markers, which are evolutionarily stable structures, act as effective indicators of broad

Table 5 Genomic sequencing and quality data of *Brucella* isolates

No	Total reads	Theoretical coverage	Assembly size	Contigs	Genome fraction (%)	L50	N50	GC (%)	MLST Type
22RB24664	8.258.186	372	3.262.665	27	98,217	2	620.005	57,24	3
22RB24665	9.763.154	440	3.263.059	34	98,201	3	391.189	57,24	3
22RB24667	9.875.300	445	3.244.153	25	97,702	3	391.739	57,25	2
22RB24672	9.881.948	445	3.261.394	27	98,173	3	391.691	57,24	2
22RB24674	11.316.846	510	3.286.198	30	97,701	3	391.747	57,14	2
22RB24676b	6.250.240	281	3.261.507	27	98,189	3	391.737	57,24	2
22RB24677	11.465.642	516	3.262.339	30	98,172	3	392.022	57,24	2
22RB24678	10.339.524	466	3.262.022	34	98,188	3	391.091	57,24	2
22RB24679	9.810.592	442	3.244.315	25	97,692	3	391.778	57,25	2
22RB24681	7.503.942	338	3.244.067	25	97,702	3	391.739	57,25	2
22RB24682	8.760.566	394	3.263.052	38	98,168	3	391.341	57,24	2
22RB24683	10.916.700	492	3.262.699	33	98,164	3	391.333	57,24	2
22RB24666	10.432.846	470	3.292.055	33	99,285	4	299.290	57,25	8
22RB24668	8.887.416	400	3.292.247	34	99,285	4	359.027	57,25	8
22RB24669	11.527.542	519	3.291.656	34	99,266	4	299.291	57,25	8
22RB24670	10.963.852	494	3.291.352	34	99,263	4	299.284	57,24	102
22RB24671	10.894.602	491	3.291.457	34	99,263	4	299.287	57,24	102
22RB24673	9.640.296	434	3.291.593	35	99,281	4	299.283	57,24	8
22RB24675	9.245.860	416	3.291.143	32	99,265	4	299.268	57,24	102
22RB24684b	5.863.356	264	3.292.527	37	99,268	4	299.259	57,25	8
22RB24685	2.651.264	119	3.292.544	35	99,278	4	299.291	57,25	8
22RB24686	11.758.676	530	3.292.198	34	99,281	4	299.259	57,25	8
22RB24687b	3.716.026	167	3.292.194	34	99,278	4	299.259	57,25	8

evolutionary patterns. Therefore, SNP analysis based on whole genomes is an ideal technique for distinguishing between *Brucella* species with highly conserved genomes [37, 39]. *B. melitensis* comprises of five main lineages (I, II, III, IV, V), with genotype II being associated with the Eastern Mediterranean lineage and containing nine different subgenotypes [40, 41]. In the present study, a WGS-SNP-based phylogenetic tree, constructed using 11 local *B. melitensis* isolates, revealed that all of the isolates are clustered in subgenotype IIb of the Eastern Mediterranean lineage. Although there are no current and comprehensive molecular epidemiological study of *B. melitensis* animal isolates representing all regions of Türkiye, the findings of this study are confirmed by those of Akar and Erganiş [42], who reported that 50 *B. melitensis* strains isolated from different farms located in seven geographical regions of Türkiye were associated with the Eastern Mediterranean lineage. Similarly, Dadar et al. [23] conducted a study analyzing 37 *B. melitensis* isolates in Iran and found that five isolates (13.5%) belonged to the American (V) lineage, with 32 isolates (86.5%) being from the Eastern Mediterranean (II) lineage. In this study, WGS data was used to perform in silico MLST analysis, revealing that isolates clustered in genotype IIe/f shared similarities with strains from Türkiye, Syria, Afghanistan, and Kuwait. All 11 *B. melitensis* isolates analyzed in the present study were found to be of subgenotype IIb, with MLST profiles identified as ST8 ($n=9$) and ST102 ($n=2$). These genotypes, ST8 and ST102, have also been identified in other studies conducted in Türkiye [42, 43].

B. abortus strains are classified into three main clades: A, B, and C (C1, C2). While clades A and B have a limited distribution (Africa), clade C is further divided into two subclades, C1 and C2, with a wide geographic distribution (six continents) [44]. There is limited information in national studies regarding the diversity, distribution, and molecular epidemiology of *B. abortus* strains [9, 45, 46], and no report was located that considers the WGS-based genetic diversity of animal *B. abortus* isolates in Türkiye. In the present study, a WGS-SNP-based analysis of 12 local *B. abortus* isolates revealed that they all belonged to subclade C1. In epidemiological studies conducted with *B. abortus* strains, it has been reported that clade C exhibited global distribution through ancient trade routes [44]. Liu et al. [47] conducted a comprehensive study investigating the genetic relatedness of *Brucella* strains in countries along the Silk Road trade route and identified a common genotype shared by strains from

Table 6 Pairwise WGS-SNP distances of *B. melitensis* strains isolated from various regions of Türkiye and originating from different regions around the world (the numbers marked in red indicate the thesis study isolates)

SNP-dists 0.7.0	22RB24666	22RB24668	22RB24669	22RB24670	22RB24671	22RB24673	22RB24675	22RB24684b	22RB24685	22RB24686	22RB24687b	63-9-BMN_EastMed._GT-IIe	66-59_EastMed._GT-IIe	B-REF-BM1-16M_America_GT-V	B-REF-BM1-Rev1_America_GT-V	B-REF-BM3-Ether_WestMed._GT-I	B115_America_GT-IV	BG2-S27_EastMed._GT-IIf	CNGB1076_America_GT-V	CNGB1120_America_GT-V	CNGB290_America_GT-V	Ether-BMT_WestMed._GT-I	F1-06B10_Africa_GT-III	F10-05-2_America_GT-IV	F10-06-16_EastMed._GT-IId	F15-06-7_WestMed._GT-I	F2-06-6_EastMed._GT-IIf	F3-02_America_GT-IV	F5-07-239A-SRR641713	F6-05-6__EastMed._GT-IIe	F8-01-155_EastMed._GT-IIb	F9-05_EastMed._GT-IIb	Reference	UK14-06_Africa_GT-III	UK19-04_Africa_GT-III	UK22-04_Africa_GT-III	UK22-06_EastMed._GT-IIb	UK23-06_Africa_GT-III	UK29-05_EastMed._GT-IIc	UK3-06_EastMed._GT-IIa	UK31-99_WestMed._GT-I	UK37-05_EastMed._GT-IIb	Uk24-06_Africa_GT-III
22RB24666	0	45	42	262	269	51	265	231	65	227	227	410	418	2309	2317	2697	2277	432	2295	2293	2297	2693	2419	2309	435	2798	412	2299	2766	410	252	253	2522	2347	2351	2379	230	2366	400	580	2802	100	2385
22RB24668	45	0	25	251	258	34	254	220	28	216	216	399	407	2298	2306	2686	2266	421	2284	2282	2286	2682	2408	2298	444	2787	401	2288	2755	399	241	242	2511	2336	2340	2368	219	2355	389	569	2791	89	2374
22RB24669	42	25	0	248	255	19	251	209	37	213	213	396	396	2287	2295	2675	2257	418	2281	2279	2283	2697	2397	2287	413	2784	398	2285	2755	396	238	239	2508	2325	2329	2365	208	2344	378	558	2788	86	2363
22RB24670	262	251	248	0	43	257	97	243	271	239	239	392	400	2289	2297	2677	2257	414	2275	2273	2277	2673	2399	2289	417	2778	394	2279	2746	392	234	235	2502	2327	2331	2359	228	2346	382	562	2782	244	2365
22RB24671	269	258	255	43	0	264	104	250	278	246	246	399	407	2296	2304	2684	2264	421	2282	2280	2284	2680	2406	2296	424	2785	401	2286	2753	399	241	242	2509	2334	2338	2366	235	2353	389	569	2789	251	2372
22RB24673	51	34	19	257	264	0	260	226	54	222	222	405	413	2304	2312	2692	2272	427	2290	2288	2292	2688	2414	2304	430	2793	407	2294	2761	405	247	248	2517	2342	2346	2374	225	2361	395	575	2797	95	2380
22RB24675	265	254	251	97	104	260	0	246	274	242	242	395	403	2292	2300	2680	2260	417	2278	2276	2280	2676	2402	2292	420	2781	397	2282	2749	395	237	238	2505	2330	2334	2362	231	2349	385	565	2785	247	2368
22RB24684b	231	220	209	243	250	226	246	0	232	4	4	391	391	2280	2288	2670	2248	413	2274	2272	2276	2674	2392	2280	408	2779	393	2278	2747	391	233	234	2501	2324	2328	2360	203	2339	373	553	2783	213	2358
22RB24685	65	28	37	271	278	54	274	232	0	236	236	419	419	2310	2318	2698	2278	441	2304	2302	2306	2702	2420	2310	436	2807	428	2307	2775	421	259	262	2531	2348	2352	2380	231	2367	401	581	2811	109	2386
22RB24686	227	216	213	239	246	222	242	4	236	0	0	387	395	2284	2292	2674	2252	409	2270	2268	2272	2670	2396	2284	412	2775	389	2274	2743	387	229	230	2497	2324	2328	2356	207	2343	377	557	2779	209	2362
22RB24687b	227	216	213	239	246	222	242	4	236	0	0	387	395	2284	2292	2674	2252	409	2270	2268	2272	2670	2396	2284	412	2775	389	2274	2743	387	229	230	2497	2324	2328	2356	207	2343	377	557	2779	209	2362
63-9-BMN-EastMed.GT-IIe	410	399	396	392	399	405	395	391	419	387	387	0	76	2249	2257	2639	2217	226	2235	2233	2237	2639	2361	2249	3	2744	394	2244	2712	12	0	382	383	2462	2289	2293	2321	208	2308	266	524	2748	392	2325
66-59-EastMed.GT-IIe	418	407	396	400	407	413	403	391	419	395	395	76	0	2253	2261	2643	2221	234	2247	2245	2249	2647	2365	2253	3	2753	403	2253	2721	20	390	391	2474	2293	2297	2325	231	2312	266	524	2756	400	2329
B-REF-BM1-16M-AmericaGT-V	2309	2298	2287	2289	2296	2304	2292	2280	2310	2284	2284	2249	2253	0	188	2763	416	2275	254	252	258	2767	1621	448	2270	2872	2255	446	2840	2249	2281	2280	223	1550	1551	1589	2267	1568	2235	2257	2878	2291	1589

Table 6 (continued)

B-REF-BM1-Rev1 America GT-V	2317	2306	2295	2297	2304	2312	2300	2288	2318	2292	2292	2257	2261	188	0	2771	424	2283	264	262	266	2775	1629	456	2278	2880	2263	454	2848	2257	2289	2290	411	1558	1559	1597	2275	1572	2243	2265	2886	2299	1597
B-REF-BM3-Ether WestMed. GT-I	2697	2686	2675	2677	2684	2692	2680	2670	2698	2674	2674	2643	2643	2763	2771	0	2731	2664	2757	2755	2751	4	2878	2763	2659	554	2645	2761	522	2643	2669	2670	2984	2807	2810	2846	2655	2823	2625	2647	626	2679	2846
B115 America GT-IV	2277	2266	2255	2257	2264	2272	2260	2248	2278	2252	2252	2217	2221	416	424	2731	0	2243	410	408	412	2735	1589	162	2238	2840	2223	88	2808	2217	2249	2250	639	1518	1519	1557	2235	1536	2203	2225	2846	2259	1557
BG2-S27 EastMed. GT-IIf	432	421	418	414	421	427	417	413	441	409	409	226	234	2275	2283	2664	2243	0	2261	2259	2263	2660	2387	2275	295	2766	226	2265	2734	226	404	405	2488	2315	2319	2347	400	2334	288	544	2770	414	2353
CNGB1076 America GT-V	2295	2284	2281	2275	2282	2290	2278	2274	2304	2270	2270	2235	2247	254	264	2757	410	2261	0	126	244	2753	1615	442	2264	2858	2241	432	2826	2235	2267	2268	469	1544	1545	1575	2259	1562	2229	2251	2864	2277	1583
CNGB1120 America GT-V	2293	2282	2279	2273	2280	2288	2276	2272	2302	2268	2268	2233	2245	252	262	2755	408	2259	126	0	242	2751	1613	440	2262	2856	2239	430	2824	2233	2265	2266	467	1542	1543	1573	2259	1560	2227	2249	2862	2275	1581
CNGB290 America GT-V	2297	2286	2283	2277	2284	2292	2280	2276	2306	2272	2272	2237	2249	258	266	2751	412	2263	244	242	0	2747	1617	444	2266	2852	2243	434	2820	2237	2269	2270	473	1546	1547	1577	2263	1564	2231	2253	2858	2279	1585
Ether-BMT WestMed. GT-I	2693	2682	2679	2673	2680	2688	2676	2674	2702	2670	2670	2639	2647	2767	2775	4	2735	2660	2753	2751	2747	0	2882	2767	2663	550	2641	2757	518	2639	2665	2666	2980	2811	2814	2842	2659	2827	2629	2651	622	2675	2850
F1-06B10 Africa GT-III	2419	2408	2397	2399	2406	2414	2402	2392	2420	2396	2396	2361	2365	1621	1629	2878	1589	2387	1615	1613	1617	2882	0	1621	2382	2983	2367	1619	2955	2361	2391	2392	1842	961	964	998	2377	979	2347	2369	2991	2401	998
F10-05-2 America GT-IV	2309	2298	2287	2289	2296	2304	2292	2280	2310	2284	2284	2249	2253	448	456	2763	162	2275	442	440	444	2767	1621	0	2270	2872	2255	192	2840	2249	2281	2282	671	1550	1551	1589	2267	1568	2235	2257	2878	2291	1589
F10-06-16 EastMed. GT-IId	435	424	413	417	424	430	420	408	436	412	412	273	273	2270	2278	2659	2238	295	2264	2262	2266	2663	2382	2270	0	2768	275	2268	2736	273	407	408	2491	2310	2314	2350	395	2329	283	541	2772	417	2348
F15-06-7 WestMed. GT-I	2798	2787	2784	2778	2785	2793	2781	2779	2807	2775	2775	2744	2752	2872	2880	554	2840	2766	2858	2856	2852	550	2983	2872	2768	0	2746	2862	112	2744	2770	2771	3085	2912	2915	2943	2762	2928	2734	2756	726	2780	2951
F2-06-6 EastMed. GT-IIf	412	401	398	394	401	407	397	393	421	389	389	206	214	2255	2263	2645	2223	226	2241	2239	2243	2641	2367	2255	275	2746	0	2245	2714	206	384	385	2468	2295	2299	2327	380	2314	268	526	2750	394	2333
F3-02 America GT-IV	2299	2288	2285	2279	2286	2294	2282	2278	2308	2274	2274	2239	2251	446	454	2761	88	2265	432	430	434	2757	1619	192	2268	2862	2245	0	2830	2239	2271	2272	661	1548	1549	1579	2265	1566	2233	2255	2868	2281	1587
F5-07-239A-SRR641713	2766	2755	2752	2746	2753	2761	2749	2747	2775	2743	2743	2712	2720	2840	2848	522	2808	2734	2826	2824	2820	518	2955	2840	2736	112	2714	2830	0	2712	2738	2739	3053	2884	2887	2915	2730	2900	2702	2724	694	2748	2923

Table 6 (continued)

F6-05-6 EastMed. GT-IIe	410	399	396	392	399	405	395	391	419	387	387	0	76	2249	2257	2643	2217	226	2235	2233	2237	2639	2361	2249	273	2744	206	2239	2712	0	382	383	2462	2289	2293	2319	378	2308	266	524	2748	392	2325
F8-01-155 EastMed. GT-IIb	252	241	238	234	241	247	237	233	261	229	229	382	390	2281	2289	2669	2249	404	2267	2265	2269	2665	2391	2281	407	2770	384	2271	2738	382	0	107	2494	2319	2323	2351	220	2338	372	552	2774	234	2357
F9-05 EastMed. GT-IIb	253	242	239	235	242	248	238	234	262	230	230	383	391	2280	2290	2670	2250	405	2268	2266	2270	2666	2392	2282	408	2771	385	2272	2739	383	107	0	2493	2320	2324	2352	221	2339	373	553	2775	235	2358
Reference	2522	2511	2508	2502	2509	2517	2505	2501	2531	2497	2497	2462	2474	223	411	2984	639	2488	469	467	473	2980	1842	671	2491	3085	2468	661	3053	2462	2494	2493	0	1771	1772	1802	2488	1789	2454	2476	3091	2504	1810
UK14-06 Africa GT-III	2347	2336	2325	2327	2334	2342	2330	2320	2348	2324	2324	2289	2293	1550	1558	2807	1518	2315	1544	1542	1546	2811	961	1550	2310	2912	2295	1548	2884	2289	2319	2320	1771	0	213	681	2305	114	2275	2297	2920	2329	635
UK19-04 Africa GT-III	2351	2340	2329	2331	2338	2346	2334	2324	2352	2328	2328	2293	2297	1551	1559	2810	1519	2319	1545	1543	1547	2814	964	1551	2314	2915	2299	1549	2887	2293	2323	2324	1772	213	0	684	2309	231	2279	2301	2921	2333	638
UK22-04 Africa GT-III	2379	2368	2365	2359	2366	2374	2362	2360	2388	2356	2356	2319	2331	1589	1597	2846	1557	2347	1575	1573	1577	2842	998	1589	2350	2943	2327	1579	2915	2319	2351	2352	1802	681	684	0	2345	699	2315	2337	2951	2361	718
UK22-06 EastMed. GT-IIb	230	219	208	228	235	225	231	203	231	207	207	378	378	2267	2275	2655	2235	400	2259	2259	2263	2659	2377	2267	395	2762	380	2265	2730	378	220	221	2488	2305	2309	2345	0	2324	360	540	2768	212	2343
UK23-06 Africa GT-III	2366	2355	2344	2346	2353	2361	2349	2339	2367	2343	2343	2308	2312	1568	1572	2823	1536	2334	1562	1560	1564	2827	979	1568	2329	2928	2314	1566	2900	2308	2338	2339	1789	114	231	699	2324	0	2294	2316	2936	2348	653
UK29-05 EastMed.GT-IIc	400	389	378	382	389	395	385	373	401	377	377	266	266	2235	2243	2625	2203	288	2229	2227	2231	2629	2347	2235	283	2734	268	2233	2702	266	372	373	2454	2275	2279	2315	360	2294	0	504	2738	382	2313
UK3-06 EastMed.GT-IIa	580	569	558	562	569	575	565	553	581	557	557	524	524	2257	2265	2647	2225	544	2251	2249	2253	2651	2369	2257	541	2756	526	2255	2724	524	552	553	2476	2297	2301	2337	540	2316	504	0	2760	562	2335
UK31-99 WestMed.GT-I	2802	2791	2788	2782	2789	2797	2785	2783	2811	2779	2779	2748	2756	2878	2886	626	2846	2770	2864	2862	2858	622	2991	2878	2772	726	2750	2868	694	2748	2774	2775	3091	2920	2921	2951	2768	2936	2738	2760	0	2784	2959
UK37-05 EastMed.GT-IIb	100	89	86	244	251	95	247	213	109	209	209	392	400	2291	2299	2679	2259	414	2277	2275	2279	2675	2401	2291	417	2780	394	2281	2748	392	234	235	2504	2329	2333	2361	212	2348	382	562	2784	0	2367
Uk24-06 Africa GT-III	2385	2374	2363	2365	2372	2380	2368	2358	2386	2362	2362	2325	2329	1589	1597	2846	1557	2353	1583	1581	1585	2850	998	1589	2348	2951	2333	1587	2923	2325	2357	2358	1810	635	638	718	2343	653	2313	2335	2959	2367	0

Table 7 Pairwise WGS-SNP distances of *B. abortus* strains isolated from various regions of Türkiye and originating from different regions around the world (the numbers marked in red indicate the thesis study isolates)

SNP-dists 0.7.0	20Y0140_Iran	20Y0153_Iran	20Y0156_Iran	22RB24664	22RB24665	22RB24667	22RB24672	22RB24674	22RB24676b	22RB24677	22RB24678	22RB24679	22RB24681	22RB24682	22RB24683	Reference	NI150_UK	18RB17257_Egypt	18RB17255_Egypt	18RB17243_Egypt	5842_Egypt	5311_Egypt	21900_Egypt	15697_Egypt	7429_Egypt	RB51-3_Egypt	RB51-4_Egypt	B11-0574_USA	B93-1491_USA	67-93_Iraq	BJ1_China	CIIMS-NV-4_India	BAU21 S4023 Bangladesh
20Y0140_Iran	0	1185	1195	1214	1214	1187	1175	1187	1190	1175	1194	1186	1187	1177	1178	176	71	78	77	77	168	77	156	79	77	77	168	99	85	1136	1187	77	1153
20Y0153_Iran	1185	0	250	269	269	132	270	132	285	270	289	131	132	272	273	1203	1194	1201	1200	1200	1195	1200	1196	1202	1200	1200	1195	1196	1200	601	282	1192	806
20Y0156_Iran	1195	250	0	249	249	250	278	250	293	278	297	249	250	280	281	1213	1204	1211	1210	1210	1205	1210	1206	1212	1210	1210	1205	1206	1210	609	290	1202	812
22RB24664	1214	269	249	0	0	269	297	269	312	297	316	268	269	299	300	1232	1223	1230	1229	1229	1224	1229	1225	1231	1229	1229	1224	1225	1229	628	309	1221	833
22RB24665	1214	269	249	0	0	269	297	269	312	297	316	268	269	299	300	1232	1223	1230	1229	1229	1224	1229	1225	1231	1229	1229	1224	1225	1229	628	309	1221	833
22RB24667	1187	132	250	269	269	0	270	6	285	270	289	131	0	272	273	1205	1196	1203	1202	1202	1197	1202	1198	1204	1202	1202	1197	1198	1202	601	282	1194	806
22RB24672	1175	270	278	297	297	270	0	270	167	0	171	269	270	24	25	1193	1184	1191	1190	1190	1185	1190	1186	1192	1190	1190	1185	1186	1190	589	164	1182	794
22RB24674	1187	132	250	269	269	6	270	0	285	270	289	131	6	272	273	1205	1196	1203	1202	1202	1197	1202	1198	1204	1202	1202	1197	1198	1202	601	282	1194	806
22RB24676b	1190	285	293	312	312	285	167	285	0	167	22	284	285	169	170	1208	1199	1206	1205	1205	1200	1205	1201	1207	1205	1205	1200	1201	1205	604	173	1197	809
22RB24677	1175	270	278	297	297	270	0	270	167	0	171	269	270	24	25	1193	1184	1191	1190	1190	1185	1190	1186	1192	1190	1190	1185	1186	1190	589	164	1182	794
22RB24678	1194	289	297	316	316	289	171	289	22	171	0	288	289	173	174	1212	1203	1210	1209	1209	1204	1209	1205	1211	1209	1209	1204	1205	1209	608	177	1201	813
22RB24679	1186	131	249	268	268	131	269	131	284	269	288	0	131	271	272	1204	1195	1202	1201	1201	1196	1201	1197	1203	1201	1201	1196	1197	1201	600	281	1193	805
22RB24681	1187	132	250	269	269	0	270	6	285	270	289	131	0	272	273	1205	1196	1203	1202	1202	1197	1202	1198	1204	1202	1202	1197	1198	1202	601	282	1194	806
22RB24682	1177	272	280	299	299	272	24	272	169	24	173	271	272	0	1	1195	1186	1193	1192	1192	1187	1192	1188	1194	1192	1192	1187	1188	1192	591	166	1184	796
22RB24683	1178	273	281	300	300	273	25	273	170	25	174	272	273	1	0	1196	1187	1194	1193	1193	1188	1193	1189	1195	1193	1193	1188	1189	1193	592	167	1185	797
Reference	176	1203	1213	1232	1232	1205	1193	1205	1208	1193	1212	1204	1205	1195	1196	0	185	192	191	191	20	191	188	193	191	191	20	187	191	1154	1205	183	1171
NI150_UK	71	1194	1204	1223	1223	1196	1184	1196	1199	1184	1203	1195	1196	1186	1187	185	0	51	50	50	177	50	165	52	50	50	177	108	94	1145	1196	86	1162
18RB17257_Egypt	78	1201	1211	1230	1230	1203	1191	1203	1206	1191	1210	1202	1203	1193	1194	192	51	0	1	1	184	1	172	3	11	1	184	115	101	1152	1203	93	1169
18RB17255_Egypt	77	1200	1210	1229	1229	1202	1190	1202	1205	1190	1209	1201	1202	1192	1193	191	50	1	0	0	183	0	171	2	10	0	183	114	100	1151	1202	92	1168

Table 7 (continued)

18RB17243_Egypt	77	1200	1210	1229	1229	1202	1190	1202	1205	1190	1209	1201	1202	1192	1193	191	50	1	0	0	183	0	171	2	10	0	183	114	100	1151	1202	92	1168
5842_Egypt	168	1195	1205	1224	1224	1197	1185	1197	1200	1185	1204	1196	1197	1187	1188	20	177	184	183	183	0	183	180	185	183	183	2	179	183	1146	1197	175	1163
5311_Egypt	77	1200	1210	1229	1229	1202	1190	1202	1205	1190	1209	1201	1202	1192	1193	191	50	1	0	0	183	0	171	2	10	0	183	114	100	1151	1202	92	1168
21900_Egypt	156	1196	1206	1225	1225	1198	1186	1198	1201	1186	1205	1197	1198	1188	1189	188	165	172	171	171	180	171	0	173	171	171	180	167	171	1147	1198	163	1164
15697_Egypt	79	1202	1212	1231	1231	1204	1192	1204	1207	1192	1211	1203	1204	1194	1195	193	52	3	2	2	185	2	173	0	12	2	185	116	102	1153	1204	94	1170
7429_Egypt	77	1200	1210	1229	1229	1202	1190	1202	1205	1190	1209	1201	1202	1192	1193	191	50	11	10	10	183	10	171	12	0	10	183	114	100	1151	1202	92	1168
RB51-3_Egypt	77	1200	1210	1229	1229	1202	1190	1202	1205	1190	1209	1201	1202	1192	1193	191	50	1	0	0	183	0	171	2	10	0	183	114	100	1151	1202	92	1168
RB51-4_Egypt	168	1195	1205	1224	1224	1197	1185	1197	1200	1185	1204	1196	1197	1187	1188	20	177	184	183	183	2	183	180	185	183	183	0	179	183	1146	1197	175	1163
B11-0574_USA	99	1196	1206	1225	1225	1198	1186	1198	1201	1186	1205	1197	1198	1188	1189	187	108	115	114	114	179	114	167	116	114	114	179	0	114	1147	1198	106	1164
B93-1491_USA	85	1200	1210	1229	1229	1202	1190	1202	1205	1190	1209	1201	1202	1192	1193	191	94	101	100	100	183	100	171	102	100	100	183	114	0	1151	1202	81	1168
67-93_Iraq	1136	601	609	628	628	601	589	601	604	589	608	600	601	591	592	1154	1145	1152	1151	1151	1146	1151	1147	1153	1151	1151	1146	1147	1151	0	601	1143	756
BJ1_China	1187	282	290	309	309	282	164	282	173	164	177	281	282	166	167	1205	1196	1203	1202	1202	1197	1202	1198	1204	1202	1202	1197	1198	1202	601	0	1194	806
CIIMS-NV-4_India	77	1192	1202	1221	1221	1194	1182	1194	1197	1182	1201	1193	1194	1184	1185	183	86	93	92	92	175	92	163	94	92	92	175	106	81	1143	1194	0	1160
BAU21_S4023_Bangladesh	1153	806	812	833	833	806	794	806	809	794	813	805	806	796	797	1171	1162	1169	1168	1168	1163	1168	1164	1170	1168	1168	1163	1164	1168	756	806	1160	0

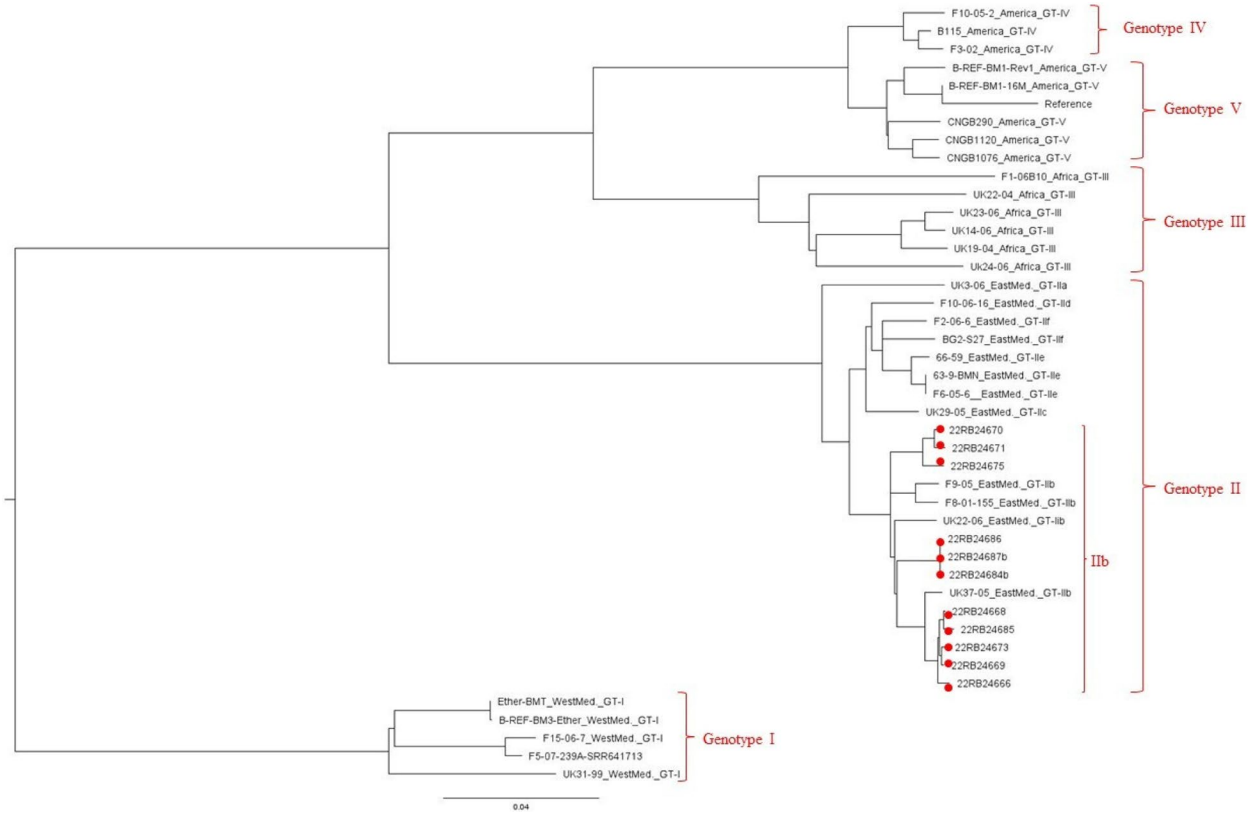

Fig. 2 Phylogenetic tree of WGS-SNP-based *B. melitensis* strains (the numbers marked in red indicate the thesis study isolates)

Türkiye, China, Kazakhstan, Italy, and Mongolia. In the present study, *B. abortus* isolates branched within subclade C1 alongside strains originating from Iran, China, and Iraq. Dadar et al. (2023) performed in silico MLST analysis of three *B. abortus* strains isolated in Iran, including one human and two bovine isolates, identifying the bovine isolates as genotype ST2 and the human isolate as genotype ST1 [23]. In the present study, 83.33% of the analyzed bovine *B. abortus* isolates were found to be genotype ST2, with two isolates being genotype ST3. To our knowledge, this is the first MLST analysis of local *B. abortus* isolates ($n=12$) in Türkiye.

Brucella species are characterized at the biotype level based on the scheme of biotyping, which distinguishes between *B. melitensis*, *B. abortus*, and *B. suis* species and subspecies [11]. In the present study, 23 *Brucella* isolates were identified at the biotype level, according to the biotyping scheme. The findings were evaluated in conjunction with the phylogenetic tree constructed based on WGS-SNP analysis, in which 11 *B. abortus* isolates were biotyped as *B. abortus* bv 3, while one isolate (22RB24676) was biotyped as *B. abortus* bv 9. Eight out of nine *B. melitensis* isolates were biotyped as *B. melitensis* bv 3, one isolate was bv 1, and one ram isolate was biotyped as streptomycin-resistant *B. melitensis* bv. *B. melitensis* bv 2, which is a rarely reported biotype in Türkiye [42, 48]. The predominant *Brucella* biotypes in Türkiye are *B. melitensis* bv 3 and *B. abortus* bv 3, while other biotypes are detected at lower rates [9, 23, 48–50]. To our knowledge, this is the first study in which streptomycin-resistant *B. melitensis* bv 2 has been isolated from a ram in Türkiye. Genotyping studies it has been reported that genotypes and biovars are not related to each other [44]. In the presented study, while the isolates showed a homogeneous distribution on a macro scale in terms of genotype, a relationship between genotype and biotype could not be established since they showed biovar diversity.

Reports indicate increasing resistance to antimicrobials among *Brucella* species [51, 52]. The application of routine phenotypic antimicrobial resistance (AMR) tests for *Brucella* spp. is limited due to the risk of laboratory-acquired infections and the requirement for BSL3-level laboratory facilities [11, 34, 53]. In the present study, *B. melitensis* and *B. abortus* strains were analyzed for antimicrobial resistance, both phenotypically and genotypically. Classic resistance-associated genes (*mecA*, *tetA*, *tetG*, *folA*, *ermA*, *ermB*, *ermC*, *mefA*, *msrA)* were not detected by WGS-based

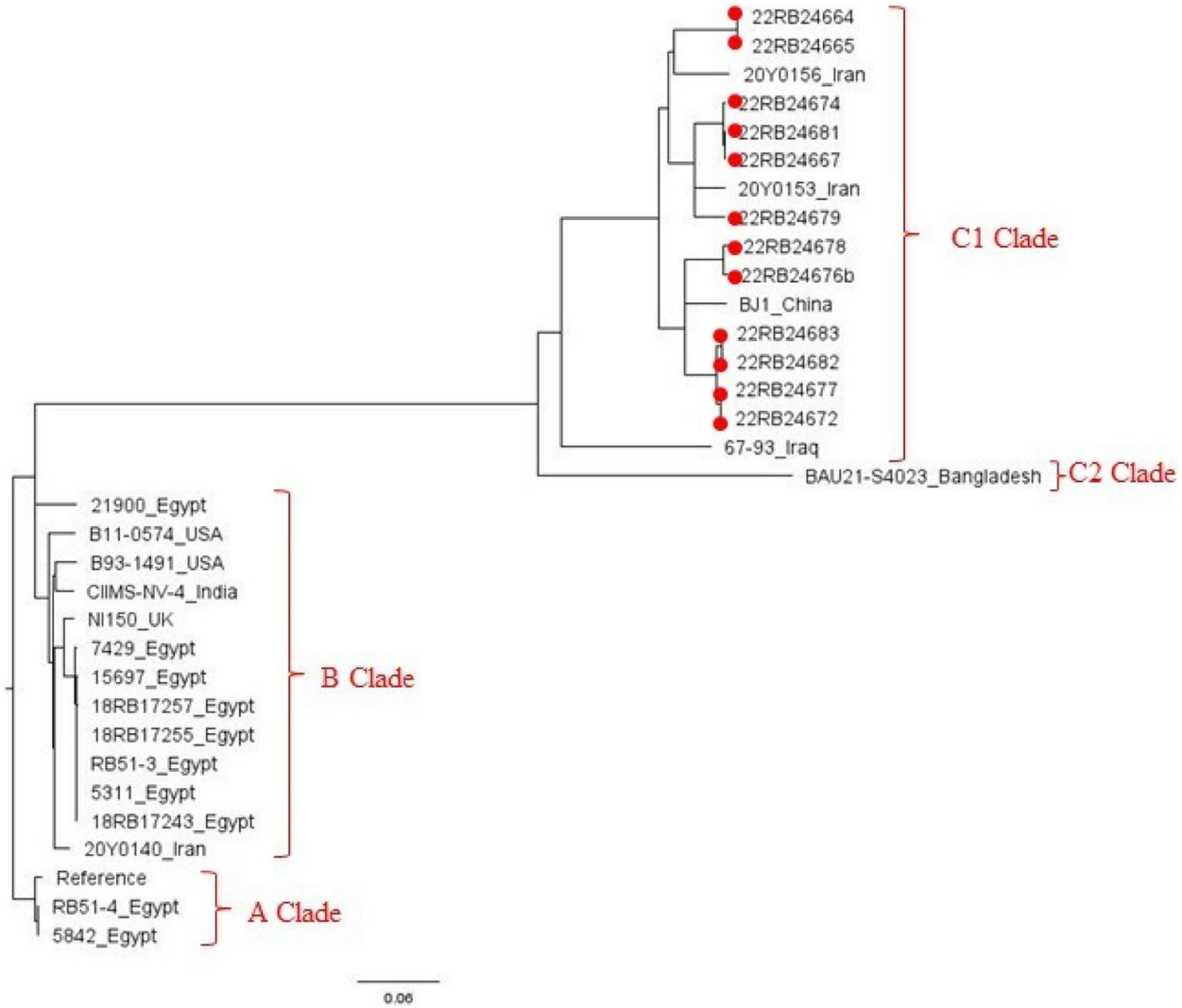

Fig. 3 Phylogenetic tree of WGS-SNP-based *B. abortus* strains (the numbers marked in red indicate the thesis study isolates)

antimicrobial resistance gene analysis. Similar findings were achieved by Dadar et al. (2023) in their WGS-based AMR gene analysis of Iranian *B. melitensis* and *B. abortus* strains. Although the researchers identified multiple peptide resistance factors (*mprF*) and genes related to efflux (*bepC*, *bepD*, *bepE*, *bepF*, *bepG*) in their study, correlation with resistance was not possible due to the presence of these genes in the genomes of both phenotypically resistant and susceptible *B. melitensis* and *B. abortus* strains [29]. Phenotypic AMR analysis was conducted using the disk diffusion method in the present study. All *Brucella* isolates were susceptible to doxycycline and gentamicin, consistent with findings from other studies [51, 52, 54–56]. A total of 45.45% of the *B. melitensis* isolates and 58.33% of the *B. abortus* isolates were resistant to rifampicin.

The higher level of resistance detected in this study to the findings of other studies conducted in Türkiye [55–57] may be due to the method used and the relatively low number of isolates. High resistance to azithromycin was detected in the present study in all *B. melitensis* isolates, and 91.66% of *B. abortus* isolates. Consistent with the findings of the current study, high rates of azithromycin resistance have been reported in the in vitro studies conducted in many countries, including Türkiye. This only emphasizes the need to consider treatment options [51, 52, 57, 58] and further underlines the importance of increasing global antimicrobial resistance [34].

Many of the associated virulence genes which have been identified and which play a critical role in the virulence of *Brucella* species are known to lack classical virulence factors and have also been shown to play an

important role in evading host immune responses [29, 59, 60]. In the present study, 43 genes related to virulence were identified. The majority of the detected genes were found to be associated with lipopolysaccharide (LPS) and the type IV secretion system (T4SS). These findings were consistent with the results of virulence gene analysis conducted by Dadar et al. [29] with Iranian strains. Wareth et al. (2021) reported the presence of the same virulence factors in all *Brucella* isolates, including those isolated from different hosts, including humans. Analysis of virulence genes through VFDB among the *Brucella* isolates obtained from cattle, sheep, goats, rams, and humans in the present study revealed no difference in the number or distribution of virulence genes related to *Brucella* species, host variation, or isolation year, and this is consistent with the findings of Wareth et al. [51] and Dadar et al. [29]. These results are to be expected when one considers the fact that there is currently no evidence of plasmid presence or genetic material exchange in *Brucella* species with highly conserved genomes [52].

Conclusion

In this study, 23 local *Brucella* isolates were examined using the WGS technique, and the genetic profiles of circulating isolates in our country were determined. The data from this study provide valuable insights into the current situation in Türkiye, while also offering the opportunity to compare with neighboring countries and contributing important epidemiological data for monitoring the global genotype distribution.

Abbreviations

AMR	Antimicrobial resistance
CARD	Comprehensive antibiotic resistance database
MLVA	Multilocus variable number of tandem repeats analyses
MLST	Multilocus sequence typing
PCR	Polymerase chain reaction
SNP	Single nucleotide polymorphism
ST	Sequence type
VFDB	Virulence factor database
WGS	Whole-genome sequencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-09921-w.

Supplementary Material 1.

Acknowledgements

In this study, we would like to express our sincere gratitude to Dr. Gamal Wareth and Dr. Hanka Brangsch for their valuable assistance.

Authors' contributions

Author Contributions: Concept –SEG and SÖ, Design – SÖ and SEG, Supervision –SÖ and SEG, Resources – SÖ and SEG, Data Collection – SÖ and SEG, Analysis and/or Interpretation – SÖ and SEG, Literature Search – SÖ, Writing – SÖ, Critical Review – SÖ and SEG.

Authors' information

This study was conducted by Songül Ötkün under the supervision of Sevil Erdenliğ Gürbilek as a doctoral thesis.

Funding

This study was supported by the budget of project number 325-06.01-2821ERA27D within the scope of the International ERA-NET ICRAD Multi-Collaboration.

Availability of data and materials

All study data are included in the article. Raw sequencing data have been submitted to the European Nucleotide Archive (ENA). The project accession number is PRJEB76221.

Declarations

Ethics approval and consent to participate

This study was approved by the Harran University Animal Experiments Local Ethics Committee (HRÜ-HADYEK) on 16.12.2021, session number 2021/009 and decision number 01–11, in accordance with the principles of "Regulation on the Procedures and Principles of Animal Experiments Ethics Committees" under the 2nd paragraph of Article 2.

Competing interests

The authors declare no competing interests.

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Received: 27 April 2024 Accepted: 11 September 2024
Published online: 30 October 2024

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