Annals of Clinical Microbiology and Antimicrobials

RESEARCH

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Analysis of the *Brucella melitensis* epidemic in Xinjiang: genotyping, polymorphism, antibiotic resistance and tracing

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Abstract

Brucella spp. are facultative intracellular pathogens that cause zoonosis- brucellosis worldwide. There has been a trend of the re-emergence of brucellosis worldwide in recent years. The epidemic situation of brucellosis is serious in Xinjiang. To analyze the epidemic situation of *Brucella* spp. in Xinjiang among humans and animals, this study identified 144 *Brucella* isolates from Xinjiang using classical identification and 16 S rRNA sequencing. MLVA, drug resistance testing, and wgSNP detection were also performed. At the same time, analysis was conducted based on the published data of *Brucella* isolates worldwide. The results showed that the dominant species was *B. melitensis* biovar 3, which belonged to GT42 (MLVA-8 typing) and the East Mediterranean lineage. The correlation among isolates was high both in humans or animals. The isolates in Xinjiang exhibited higher polymorphism compared to other locations in China, with polymorphism increasing each year since 2010. No amikacin/kanamycin-resistant strains were detected, but six rifampicin-intermediate isolates were identified without *rpoB* gene variation. The NJ tree of the wgSNP results indicated that there were three main complexes of the *B. melitensis* epidemic in Xinjiang. Based on the results of this study, the prevention and control of brucellosis in Xinjiang should focus on *B. melitensis*, particularly strains belonging to *B. melitensis* bv.3 GT42 (MLVA-8 typing) and East Mediterranean lineage. Additionally, the rifampicin- and trimethoprim-sulfamethoxazole- resistance of isolates in Xinjiang should be closely monitored to avoid compromising the therapeutic efficacy and causing greater losses. These results provide essential data for the prevention and control of brucellosis in Xinjiang and China. Although the isolates from Xinjiang have significant characteristics among Chinese isolates and can reflect the epidemiological situation of brucellosis in China to some extent, this study cannot represent the characteristics of isolates from other regions.

Keywords *Brucella*, MLVA, Polymorphism, Minimal inhibitory concentration, SNP

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Introduction

Brucella spp. are facultative intracellular pathogens that cause the zoonosis- brucellosis. Brucellosis not only affects public health and safety but also influences animal husbandry and economic development, leading fever, fatigue and reduced labor in humans, and reproductive disorders in animals [1]. Brucellosis is epidemic in South America, the Middle East, and parts of Asia [2], with more than half a million cases annually and incidence rates exceeding 100 per million in endemic countries [3]. However, the actual incidence is estimated to be 10~25 times higher than reported, according to the data from World Health Organization (WHO) [4]. Six classical species and six new (non-classical) species have been identified based on biochemical characteristics and host preferences [5]. Among them, *B. melitensis*, *B. abortus* and *B. suis* are the primary causes of human brucellosis [5]. In China, existing results showed that *B. melitensis* biovar 1 was the dominant strain in previous years, but *B. melitensis* biovar 3 was the main isolate in recent years [6, 7].

The spread of *Brucella* spp. is facilitated by livestock trade and tourism, leading to a pattern of re-emergence and constant expansion of brucellosis [4, 8]. According to the data collected by WOAH in 2021 (https://wahis.woah.org), France, Cyprus, Croatia, Israel, Australia, and other countries reported brucellosis outbreaks from 2019 to 2022. The last reported brucellosis outbreaks in these countries occurred 10 years ago or even longer. There also has been a trend of the re-emergence of brucellosis in China in the last decade [9]. According to the data released by the Chinese Center for Disease Control and Prevention (CCDC), the numbers of newly reported brucellosis cases in China from 2020 to 2022 were 48,455, 69,767 and 65,384 (https://www.phsciencedata.cn/Share/). Previous studies showed that the main epidemic strains of *B. melitensis* in China belonged to 9 complexes, of which one isolate from Xinjiang Uygur Autonomous Region (referred to as Xinjiang in this study) was associated with all complexes [10], and an amikacin-resistant *B. abortus* strain was first isolated from Xinjiang [11]. The location of Xinjiang is very important. Xinjiang is located in northwestern China and in the hinterland of the Eurasian continent. Xinjiang borders the provinces of Qinghai, Tibet and Gansu in China, and borders Mongolia, Russia, Kazakhstan, Afghanistan, Pakistan and India. Brucellosis prevalence in these areas is relatively serious. These factors haveurged us to consider the brucellosis epidemic trend in Xinjiang.

In this study, the prevalence of *Brucella* spp. in Xinjiang was comprehensively analyzed, including genotyping, polymorphism, antibiotic resistance and tracing. Identification typing and polymorphism of *Brucella* spp. were based on conventional and molecular methods. Compared to the conventional methods (such as culturing, agglutination testing, phage typing, and biochemical analyses), molecular methods (mainly based on multiple PCR molecular typing) were shown to be rapid, relevant, and efficient for typing and clustering *Brucella* strains [12]. Multiple Locus Variable Number of Tandem Repeats Analysis (MLVA) was wildly used in typing and polymorphism of *Brucella* spp [13]. In recent years, SNP (single nucleotide polymorphism), especially wgSNP (whole genomic SNP), has been widely used for tracing of *Brucella* isolates [14, 15]. The aims of this study were as follows: (1) MLVA was used to analyze the genotype, polymorphism, and individual differences of *Brucella* isolates in Xinjiang to better understand the changes in the population; (2) amikacin/kanamycin-resistance detection was used to analyze whether the amikacin/kanamycin -resistant isolates epidemic were in ruminants and humans; (3) the minimum inhibitory concentration (MIC) values of the clinic brucellosis treatment drug for isolates were detected to analyze resistance; and (4) a phylogenetic tree was constructed with the wgSNP to analyze the relationship between the isolates in Xinjiang and those *Brucella* isolates in China and to trace these strains.

Materials and methods

The strains and genomes of *Brucella* spp. used in this study

The genome sequences of the public *B. melitensis* with information of collection date and location were downloaded from NCBI GenBank (www.ncbi.nlm.nih.gov/genome/?term=brucella) (Table S 1). The wgSNP of Chinese *Brucella* isolates involved in this study had been published previously [10]. Cultured and inactivated *Brucella* isolations used in this study were carried out in a BSL-3 laboratory of the National Reference Laboratory for Animal Brucellosis. The information of all the isolates used in this study was listed in Table S1.

Identification

Classical identification of *Brucella* strains may be performed by using a combination of the following detections [16]: CO_2 requirement test, production of H_2S (detected by lead acetate papers) test, urease and oxidase tests, the slide agglutination test with anti-A, -M or -R monospecific sera, and/ or phage Tbilisi (Tb) lysis test. In this study, positive control using *B. melitensis* bv.1 strain 16 M and negative control using saline water were established for each test.

MLVA typing

After the *Brucella* isolates from Xinjiang were identified as *Brucella* species by 16 S rRNA sequencing in our

laboratory, the biochemical characteristic detection was used to identify the biovar. The genomic DNA of these isolates was extracted using the Wizard Genomic DNA Purification kit (Promega, USA) according to the manufacturer's instructions. The primers for MLVA-16 typing were referred to the published study [17], and all primers used in this study were listed in Table S1. A phylogenetic tree using the UPGMA method was generated with BioNumerics software (http://www.applied-maths.com/bionumerics) using default software settings [6]. Meanwhile, a minimum spanning tree (MST) was also generated with BioNumerics software [10, 18].

This study also downloaded and collected published MLVA typing data from MLVA bank databases (http://mlva.i2bc.paris-saclay.fr/brucella/) and published studies [19–27] (Table S2). If multiple data points were available from the same province/site/country, the data for MLVA-16 analysis were chosen in this study.

Polymorphism analysis

The polymorphism analysis was based on the results of MLVA typing. All the results of MLVA sites were uploaded to the Comparing Partitions Website (http://www.comparingpartitions.info/index.php?link=Tut4), and polymorphism analysis was performed.

Amikacin/kanamycin-resistance detection

Based on a previous study [11], our lab established a real time PCR (qPCR) method to detect the *aph(3')-IIa* gene. The upstream primer sequence was 5'- AACTGT TCGCCAGGCTCAAG −3', and the downstream primer sequence was 5'- AAGCGGCCATTTTCCACCAT −3'. The probe sequence was 5'-VIC- TCGTGACCCATG GCGATGCCTGCTT-BHQ-3'. The specific primers for *Brucella* species were described in a published study [28]. The amplification system was 20 μL / sample, including 10 μL of premix ex Taq (probe qPCR) (Takara, Japan), 5.6 μL of ddH_2O, 0.4 μL of primers and probes (4 primers and 2 probes, the final concentration was 0.2 μM), and 2 μL of samples. For two-step amplification detection, the conditions were pre denaturation at 95 °C for 30 s, reaction for 45 cycles, 95 °C for 5 s, 60 °C for 30 s, and the corresponding fluorescence signal was collected at the end of each reaction. This method used double probes to detect amikacin/kanamycin-resistant *Brucella* strains with good specificity. The minimum *Brucella* content detected was 10^2 CFU/μL. All the primers and probes were synthesized by Sangon Biotech (Shanghai) Co., Ltd (Shanghai, China).

MIC value

According to the recommendation of the WHO and the clinical medication for brucellosis in China, rifampicin (REP), doxycycline (DOX), streptomycin (SM), trimethoprim-sulfamethoxazole (TMP/SMZ, trimethoprim: sulfamethoxazole = 1:19), ceftriaxone sodium (CRO), gentamicin (GM), levofloxacin (LfX), ampicillin (AMP) and ciprofloxacin (CIP) were selected to detect the MIC value. The susceptibility of isolates to these drugs (National Institutes for Food and Drug Control, China) was tested by the double dilution method in 96-well plates according to CLSI guidelines. Isolates were cultured on Brucella agar (BD, USA) media and incubated at 35 ± 2 ℃ for 72~96 h. Isolates were suspended in saline water to 0.5 McF turbidity and suspended in Brucella broth (BD, USA). Drugs were diluted in a 96-well plate ranging from 0.125 to 256 μg/mL. Then 100 μL dilute solution (approximately 1×10^5 CFU/well) was added. The plate was cultured at 35 ± 2 °C for 48 h. The quality control strain was *Streptococcus pneumoniae* ATCC 49619 (refer to CISL_M45 (2016)) (to test whether the drug dilution is qualified), and the positive control strain was *B. melitensis* bv.3 strain Ether.

Whole genome sequencing and SNPs identification

Library preparation of isolates was performed using the MGIEasy Universal DNA Library Kit (MGI, China) according to the manufacturer's manual. Genomic DNA was sheared randomly to construct three read-libraries with lengths of 350 bp physico-chemical methods by Beijing Genomics Institute (BGI) (Shenzhen, China). The genomes were sequenced above 100-fold (100×) genome coverage. SNPs and indels identification and annotation were performed as described in a previous study [10]. Some SNPs (not all) was identified by PCR amplification and sequencing.

Clustering analyses and phylogenetic tree construction

The genome of *B. melitensis* bv.2 str. ATCC 23457 (GCA0000_22625.1) was selected as the reference. SNP calling and annotation were performed as described in a previous study [29]. PhyloSNP [30] was used to generate phylogenetic trees. The neighbor-joining (NJ) method with 1000 bootstraps was used.

Statistical analysis

Statistical analyses were performed using Excel and SPSS. A P value < 0.05 was considered significant when using one-way analysis of variance (ANOVA). The figure of MST was generated with BioNumerics software, and the figure of polymorphism of isolates in China was generated with ArcMap (version 10.2). The figure of the NJ tree based on wgSNP was exported by iTOL (https://itol.embl.de). Other figures were drawn by Excel software.

Results

All isolates belonged to *B. melitensis* biovar 3 in both humans and animals

In this study, 144 *Brucella* strains isolated from cattle and sheep in 2017~2019 were obtained. Among them, 43 strains were isolated from Tarbagatay. The numbers of isolates from Changji, Altay, Ili, Aksu, Bayingol, Kashgar and Tulufan were 16, 27, 32, 16, 6, 2 and 2, respectively. Additionally, 30 *Brucella* isolates were from cattle, and the rest were from sheep.

The results of classical identification showed that all the isolates were *B. melitensis* biovar 3. Compared with published data of isolates from human in Xinjiang [18], the dominant species was *B. melitensis* biovar 3 both in humans and animals.

The isolates exhibited high correlation in Xinjiang by MLVA typing

MLVA-8 typing. The results of MLVA were uploaded to the MLVA bank for MLVA-8 typing. The results showed that the isolates were divided into 6 genotypes (GT), which included two new genotypes named NEW1 and NEW2 (Fig. 1A). The MST showed that GT 42 was centrally located and related to other isolates. GT 63, GT 45, NEW1 and NEW2 had only one VNTR site difference when compared to GT42. There was also one VNTR site difference between GT 112 and GT 63. GT 45, NEW1 and NEW2 may vary from GT 42.

MLVA-16 typing. The results showed that the isolates were divided into 58 genotypes, including 40 genotypes that contained only one isolate. All the genotypes belonged to the East Mediterranean lineage (Fig. 1B).

The polymorphism of isolates in Xinjiang was higher compared to other locations increased year by year

The Simpson index showed that 16 VNTR sites had different degrees of polymorphism. Only two VNTR sites exhibited high polymorphism. Compared with Panel 1 (Bruce06, Bruce08, Bruce11, Bruce12, Bruce42, Bruce43, Bruce45 and Bruce55) and Panel 2 A (Bruce18, Bruce19 and Bruce21), the polymorphism of Panle 2B (Bruce04, Bruce07, Bruce09, Bruce16, Bruce30) was higher. Among them, the polymorphism of Bruce16 was highest, and the Simpson index was 0.826. The isolates from Xinjiang in this study exhibited high genetic relatedness (Table 1). Statistical polymorphism by location showed that the isolates from Altay had low polymorphism, while those from Aksu had high polymorphism.

Published data about *Brucella* isolates from Xinjiang (323 strains, including the isolates in this study) were also analyzed for polymorphism. The results showed that the VNTR sites of Bruce21, Bruce55, Bruce45 and Bruce08 had no polymorphism in isolates from Xinjiang. Compared with the polymorphism results of isolates from Xinjiang among different years, the polymorphism of *Brucella* isolates in Xinjiang increased year by year.

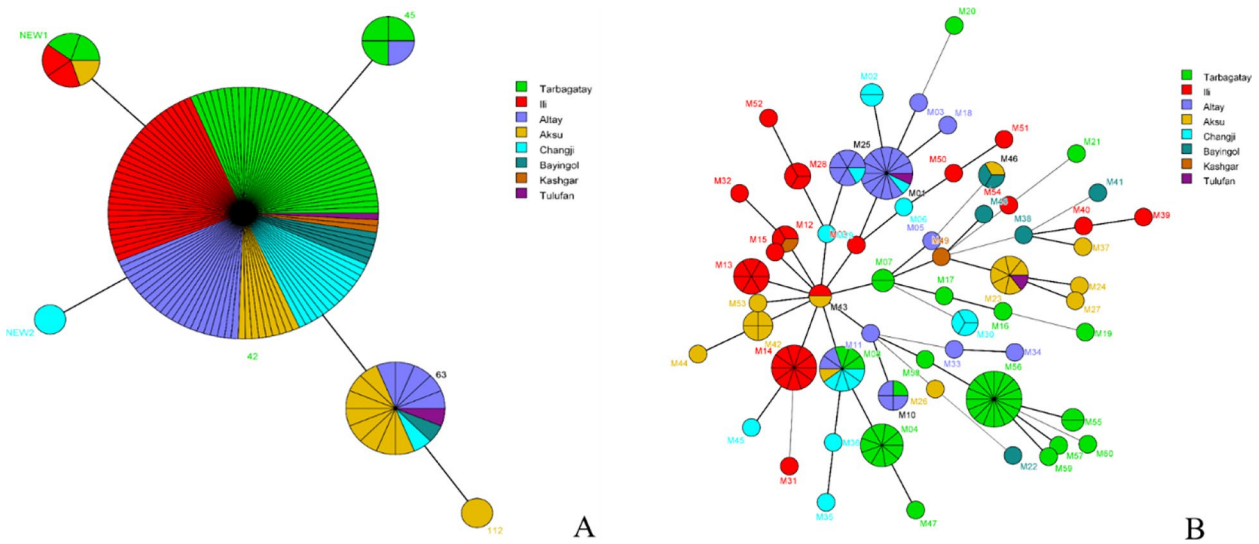

Fig. 1 MST based on the data of MLVA typing. **A** was the MST based on the data of MLVA-8 typing. **B** was the MST based on the data of MLVA-16 typing. Different colors represented different locations. The area of the circle indicated the number of strains. And the larger the area of the circle, the more strains of the genotype. Numbers and/or letters outside the circle represented genotypes. For example, in Fig. 1A, the number outside the largest circle was 42, indicating that GT 42 had the most strains, and different colors in the circle mean the locations and number of strains that composed this genotype. To distinguish from the results of MLVA-8 typing, the pre-genotype of MLVA-16 (Fig. 1B) was marked as "M"

Table 1 Simpson index of VNTR sites in Xinjiang

	Aksu	Ili	Tarbagatay	Changji	Altay	Bayingol	Tulufan	Kashgar	Total
Bruce 30	0.125	0.778	0.55	0.517	0.644	0.867	1	1	0.685
Bruce 16	0.642	0.51	0.6	0.775	0.681	0.333	1	1	0.826
Bruce 09	0.233	0.272	0.133	0.233	0.145	0.533	0	0	0.2
Bruce 07	0	0	0.512	0	0	0	0	0	0.261
Bruce 04	0.592	0.454	0.218	0.525	0.313	0.533	1	1	0.481
Bruce 21	0	0	0	0	0	0	0	0	0
Bruce 19	0.125	0.121	0.047	0.125	0.074	0.733	0	0	0.12
Bruce 18	0.458	0	0	0.325	0	0.6	0	0	0.142
Bruce 55	0	0	0	0	0	0	0	0	0
Bruce 45	0	0	0	0	0	0	0	0	0
Bruce 43	0.525	0	0	0.125	0.313	0.333	1	0	0.188
Bruce 42	0.125	0	0	0	0	0	0	0	0.014
Bruce 12	0	0	0.133	0	0.074	0	0	0	0.054
Bruce 11	0.125	0.121	0.091	0	0	0	0	0	0.068
Bruce 08	0	0	0	0	0	0	0	0	0
Bruce 06	0	0	0	0.125	0	0	0	0	0.014

This study also analyzed polymorphisms in published data from Chinese *Brucella* isolates based on the results of MLVA-16 typing (1754 strains, including the isolates in this study). The results showed that Inner Mongolia, Xinjiang, Qinghai, Ningxia and Hainan had higher polymorphism, while Guangdong had lower polymorphism among the locations with more than 50 isolates (Fig. 2).

Six rifampicin intermediate *Brucella* isolates were identified

All the isolates were tested for amikacin-resistant with qPCR based on the methods established by our laboratory, and no amikacin-resistant isolates were detected.

The drugs used in this study were double diluted from 0.125 to 256 μg/mL in 96-well plates (except for TMP/SMZ). The MIC values of *B. melitensis* bv. 3 strain Ether was used as a reference. The MIC value of isolates of REP, DOX, SM, TMP/SMZ, CRO, GM, LfX, AMP and CIP were 1~2 μg/mL, 0.06~0.12 μg/mL, 0.5~4 μg/mL, 0.03/0.06~0.24/4.8 μg/mL, 0.12~2 μg/mL, 0.015~0.06 μg/mL, 0.25~2 μg/mL, 0.5~4 μg/mL and 0.5~1 μg/mL, respectively. The MIC value of reference strain for these drugs were 0.5 μg/mL, 0.12 μg/mL, 2 μg/mL, 0.015/0.3 μg/mL, 0.5 μg/mL, 0.03 μg/mL, 0.5 μg/mL, 1 μg/mL and 0.5 μg/mL, respectively. *Brucella* isolates were sensitive to GM, SM, DOX and TMP-SMZ based on CLSI M45. According to the standard of other slow-growing bacteria of CLSI for rifampin, any MIC value ≤ 1 μg/mL was susceptible, 2 μg/mL was intermediate, and ≥ 4 μg/mL was resistant. Six rifampicin-intermediate strains were found in this study. Therefore, the highly variable sites of the *rpoB* gene were detected according to a published study [31], and no *rpoB* gene mutation was found (Fig. 3).

Three complex *Brucella* isolates were epidemic in Xinjiang

Based on the results of MLVA-16 typing, five regions with more than 5 isolates were selected for further analysis. Two isolates were randomly selected from each region. The selected strains were N2-17, N2-55, N8-7, N8-121, N9-1, N9-21, N11-12, N11-65, Y13-103 and Y13-113. The wgSNPs of 10 sequenced strains in this study, *B. melitensis* isolates around the world and standard strains of different species were used to construct the phylogenetic tree by the NJ method (Figure S1). The results showed that the eight strains sequenced in this study were clustered with the Chinese isolates, and the two strains isolated from Tarbagatay, were in the same branch as the strains isolated from Turkey, Russia, India, etc.

The 10 isolates were combined with the published Chinese isolates and SNPs involved in previous studies to construct a genome-wide NJ phylogenetic tree (Fig. 4). The results showed that the two isolates from Tarbagatay, which were closely related to foreign isolates, were not in the same branch as those from other regions in China, indicating that the isolate was only prevalent in Xinjiang. The strains isolated from Altay, Changji and Ili were in the same branch. The strains of this branch were all isolated from Xinjiang and were not in the same branch as the strains isolated from other regions. The other isolates from Bayingol and Changji were in the same branch as those from Guangdong. Another isolate from the Ili

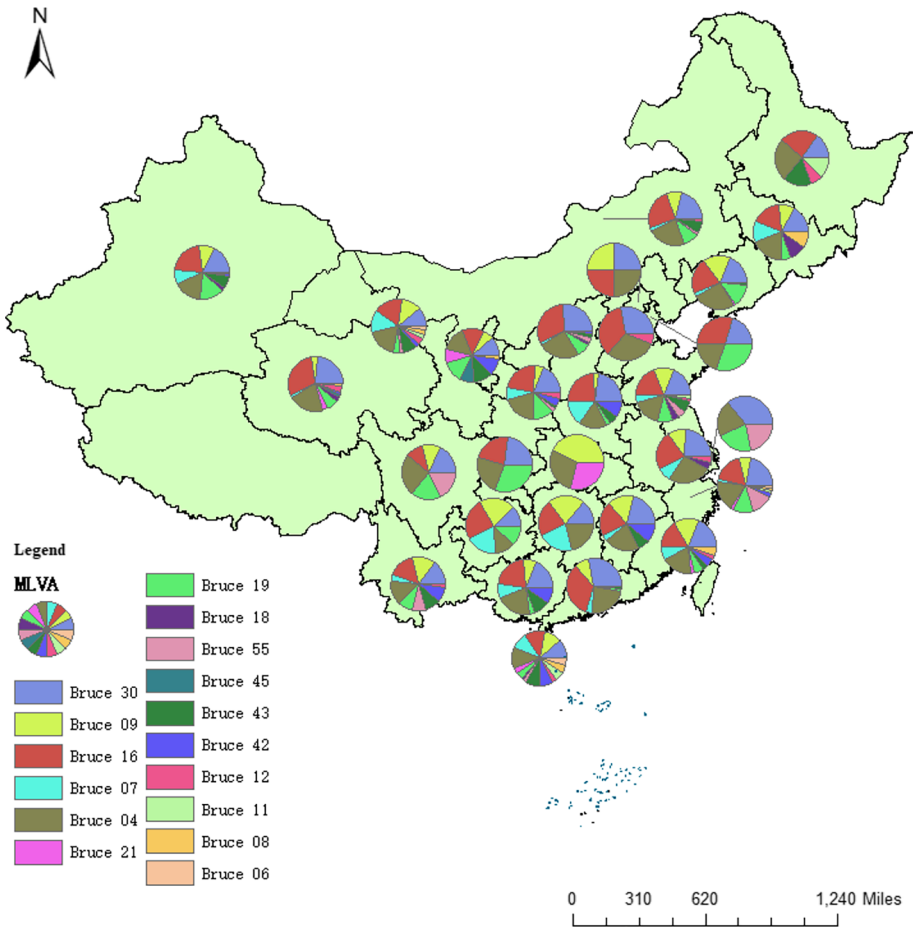

Fig. 2 The polymorphism of isolates in China based on the data of VNTR sites. Different colors represented different VNTR sites. Each circle was composed of different colors. The more colors indicated the higher polymorphism of isolates in the location, and the less colors indicated the lower polymorphism of isolates. The larger the color area in the circle, the higher polymorphism of these VNTR sites

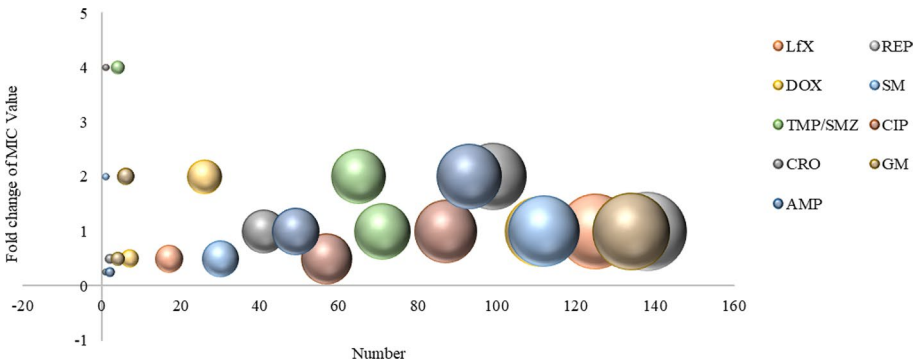

Fig. 3 Number of isolations distribution of fold change of MIC values for different drugs (Compared with reference strain of *B. melitensis* bv.3 strain Ether). Different colors represented different drugs. The area of the circle indicated the number of strains. For REF, the MIC value of reference was 0.5 μg/mL, the MIC value of isolates was 1 μg/mL, then the fold change of MIC value was 2

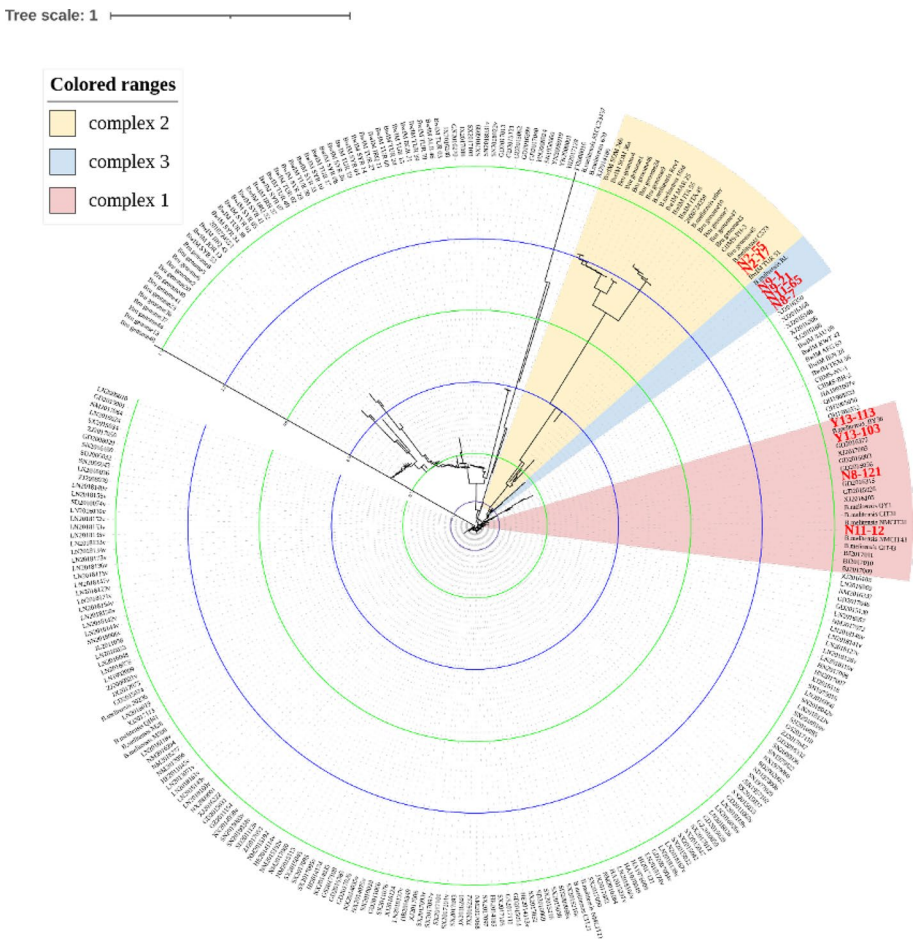

Fig. 4 NJ tree based on wgSNP of *Brucella* isolates. The red letters were the isolates sequenced in this study.The colored ranges were the complex including sequenced isolates. The length of the tree scale represented one SNP constructed the phylogenetic tree

region was in the same branch as the isolates from Inner Mongolia and Gansu and was also the closest isolate to the isolates from other regions in China. This was also the main epidemic branch in China at present.

Discussion

Based on the results of this study, the prevention and control of brucellosis in Xinjiang should focus on *B. melitensis*, particularly strains belonging to *B. melitensis* bv.3 GT42 (MLVA-8 typing) and East Mediterranean lineage. The results showed that there were several genotypes of *Brucella* isolates in China; however, GT42 was the most prevalent (MLVA-8) [7, 23]. The results of MST based on MLVA typing showed that the isolates from China were highly correlated with those from Kazakhstan and Turkey [32]. MLVA typing could be used to distinguish species, biovar or some isolates and analyze the relationships between the isolates [33]. However, it could not be used for tracing due to the risk of homology of these repeated sequences [12]. The MST results constructed by MLVA typing in this study were similar to the published studies in China [32, 34], and the isolates in Xinjiang exhibited a close relationship with those in other regions in China.

The isolates from regions that are major points of brucellosis spread in China need more attention due to their high polymorphism. The polymorphism index of isolates showed the differences in polymorphism in different regions. The higher the Simpson index, the greater the polymorphism. It was found that strains from Inner Mongolia, Xinjiang, Qinghai, Ningxia and Gansu with high polymorphism were developed in animal husbandry and endemic regions of brucellosis. Strains from Hainan, Fujian and Zhejiang, which had low polymorphism, belonged to cattle and sheep product or dairy product inflow regions (https://data.stats.gov.cn/index.htm). All of these regions belonged to the major regions or important points of the spread of brucellosis in China [6]. The regions with high polymorphism exhibited higher genetic diversity and genomic

variation of *Brucella* spp. Therefore, there was a possibility of changes or alterations in the dominant epidemic *Brucella* strains. The isolates of these regions should be taken seriously, and the frequency of surveillance needs to be increased to avoid the emergence of new variations in these regions. Further research could focus on the correlation between high polymorphism and specific factors to better understand the evolution of *B. melitensis*.

The rifampicin- and trimethoprim-sulfamethoxazole-resistance of isolates in Xinjiang should be closely monitored to avoid compromising the therapeutic efficacy and causing greater losses. This study found no transmission of amikacin/kanamycin-resistant strains was found in cattle and sheep in this study. The results of drug resistance analysis indicated that *B. melitensis* in Xinjiang was sensitive to common brucellosis treatment drugs, but there was a trend of resistance to rifampicin and trimethoprim-sulfamethoxazole (with higher MIC values). In recent years, drug resistance detection of *Brucella* spp. has been carried out worldwide, and some resistant strains have been found. Current studies have shown that rifampicin-resistant *Brucella* strains were isolated in Turkey [35], Egypt [36], Norway [37], Brazil [38], Kazakhstan [39], Qatar [40], Malaysia [41], South Korea [42], China [43], Iran [44], Italy [45] and other countries. Trimethoprim-resistant strains were detected in Brazil [38], Turkey [46], China [43] and Iran [44]. In addition, ciprofloxacin- and streptomycin-resistant strains were observed in Brazil [38], Turkey [46] and South Korea [42]. Studies of drug resistance for *Brucella* spp. were useful to analyze the variation and epidemic trends of *Brucella* under antibiotic selection pressure. At the same time, those results could guide clinical medication and promote the prevention, control, and eradication of brucellosis.

The results of genomic analysis show that isolates in China were in the same branch but not in the same branch as those in other regions of the world [15]. Chinese isolates were closely related to those in the Middle East, such as in Turkey. A study used the published genome of *Brucella* to construct an NJ tree based on wgSNP and showed that the isolates named BL from Xinjiang were in the same branch as the isolates from Turkey, Russia and other regions [19]. The phylogenetic tree based on wgSNP of two Tarbagatay isolates obtained in this study showed that these were in the same branch as the isolates from Turkey, and this branch also included the isolates from Russia, India and other regions. However, these isolates were not in the same branch as the isolates from other regions of China.

The results of the phylogenetic tree constructed in this study based on wgSNP indicated that there were mainly three complexes of the *B. melitensis* epidemic in Xinjiang. The first was the Xinjiang-specific complex, mainly epidemic in different locations in Xinjiang. The second complex was mainly related to or similar to the strains in most regions of China. The third complex was mainly similar to the isolates from Turkey and other regions. The epidemic area of this third complex in Xinjiang was limited, and it was not epidemic in other regions in China. Due to the enforcement of brucellosis detection in cross-border trade, it was speculated that foreign strains were transmitted through wild animals. A study which conducted serum detection for 258 wild ruminants in Xinjiang found that the *Brucella* spp. serum positive rate was 2.3% (95 confidence interval 0.5~4.2%) [47], which to some extent supports the results of this study. Meanwhile, the transmission of *B. melitensis* also deserves further investigation.

Conclusion

In conclusion, the dominant *Brucella* species exhibits higher polymorphism in Xinjiang than other locations in China, and there is an emerging trend of rifampicin-resistant trend in Xinjiang. This study identified 144 isolates of *Brucella* and their biovars. MLVA typing, drug resistance testing, and wgSNP detection were also performed. Additionally, analysis based on the published data of *Brucella* isolates worldwide was conducted. The results showed that the dominant species was *B. melitensis* biovar 3, and the correlation among isolates was high. Since 2010, the polymorphism of isolates in Xinjiang has been increasing each year. No reported amikacin/kanamycin-resistant strains were found, but 6 rifampicin-intermediate isolates were found without *rpoB* gene variation. The NJ tree of the wgSNP results indicated that there were three main complexes of the *B. melitensis* epidemic in Xinjiang. This study analyzes the prevalence, genotyping, diversity, drug resistance, and tracing of *B. melitensis* in Xinjiang. Based on the results of this study, the prevention and control of brucellosis in Xinjiang should focus on *B. melitensis* in both humans and animals, particularly strains belonging to *B. melitensis* bv.3 GT42 (by MLVA-8 typing) and East Mediterranean lineage. Additionally, the rifampicin- and trimethoprim-sulfamethoxazole- resistance of isolates in Xinjiang should be closely monitored to avoid compromising the therapeutic efficacy and causing greater losses.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-024-00724-0.

Supplementary Material 1.

Acknowledgements
We would like to express our deep gratitude to the staff for their effort and assistance with the strain collection at different levels in CCDC and CADC. Furthermore, we are grateful to Haijian Zhou (National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention), Qingmin Wu (College of Veterinary Medicine, China Agricultural University) and Hai Jiang (State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention) for their advices in setting up this study.

Authors' contributions
Conceptualization and study design: XWY, HJ, JBD; strains collection and identification: YL, ZJB; data analysis: XWY, LL; laboratory work: XWY, YL, NL, XWP, YHZ, XQZ, LL, ZJB; writing-review & editing: HJ, JBD. All the authors read and approved the final version of the manuscript.

Funding
This work was supported by the Innovation Program of Chinese Academy of Agricultural Sciences (No. CAAS-CSLPDCP-202403), Beijing Municipal Natural Science Foundation (No. 7222120), The Agricultural Science and Technology Innovation Program (ASTIP) (No. ASTIP-IAS-15), Central Public-interest Scientific Institution Basal Research Fund (No. 2023-YWF-ZYSQ-09), National Natural Science Foundation of China (No. 81902105), and grant from the State Key Laboratory of Infectious Disease Prevention and Control, China CDC) (No. 2021SKLID508).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests
The authors declare no competing interests.

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Received: 22 July 2023 Accepted: 1 July 2024
Published online: 10 August 2024

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