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Genomic analysis of *Brucella melitensis* isolates recovered from humans in south Tunisia over 35 years between 1988 and 2022

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Abstract

Brucella melitensis is a zoonotic pathogen that poses a worldwide public health challenge. In recent years, whole-genome sequencing has become a widely accepted molecular typing method for the genomic epidemiology of brucellosis. This study reports the genomic characteristics of 24 *B. melitensis* strains isolated from human infections in southern Tunisia over 35 years (1988–2022). We utilized WGS to analyze the clonal relationships of these strains, their relatedness to international sequences, their antimicrobial resistance determinants, and their virulence factors. Our findings revealed a high genetic stability over three decades. All isolates were identified as *B. melitensis* biovar 3 and were assigned to the same sequence type, ST11, using the MLST-9 scheme. Using the MLST-21, Tunisian sequences shared 20 out of 21 alleles and were assigned to 2 closely related STs (ST89 and ST114). Phylogenetic analysis indicated that all Tunisian sequences were grouped into a single subcluster within lineage I, the West Mediterranean clade, and were highly related to other strains from the Maghreb region (Morocco and Algeria). Antimicrobial resistance analysis revealed no classical resistance determinants. However, *mprF*, *bep*CDEFG genes, and missense mutations in *rpo*B, *gyr*A, *gyr*B, and *par*C genes were identified. Virulence analysis identified 67 genes, predominantly involved in lipopolysaccharide biosynthesis and the type IV secretion system. To our knowledge, this study represents the first genomic investigation of *B. melitensis* strains circulating in Tunisia. Our findings underscore the importance of genomic surveillance in understanding the epidemiology and evolution of brucellosis in North Africa.

Keywords *Brucella melitensis*, Tunisia, WGS, Epidemiology, Genotyping

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Introduction

Brucellosis is a zoonotic disease caused by bacteria of the genus *Brucella* that poses a significant public health challenge worldwide. The disease is most commonly transmitted to humans through the consumption of unpasteurized dairy products or direct contact with infected animals [1]. Clinically, *Brucella* infections can manifest in various forms, ranging from subclinical to acute, subacute, to chronic diseases [2, 3]. Among the species of the genus *Brucella*, *B. melitensis* is considered the leading agent involved in the majority of human infections [4].

Despite extensive efforts to eradicate brucellosis in many countries, the disease persists in regions such as the Mediterranean countries, the Balkan countries, the Middle East, parts of Africa, and some areas in Asia and Latin America [5]. In the Mediterranean region, where livestock farming plays a crucial role in the economy, the prevalence of *B. melitensis* remains notably high despite control measures [6]. This emphasizes the ongoing necessity for thorough surveillance, effective control strategies, and public health interventions to mitigate the global impact of the disease.

In Tunisia, brucellosis remains endemic in both humans and livestock and is classified as a notifiable disease under national regulations. The incidence of human brucellosis has significantly increased, rising from 2.9 cases per 100,000 inhabitants in 2008 to 9.8 cases in 2017 [7, 8]. More than 80% of notified human cases originate from south Tunisian regions including Gafsa, Kasserine, Tozeur et Kebili [9].

Traditional biotyping techniques and molecular methods, including Multi-locus Sequence Typing (MLST) and Multi-locus variable-number tandem repeat analysis (MLVA), have historically been employed to characterize and monitor *B. melitensis*. However, these approaches often require substantial time and technical expertise and provide limited resolution for *B. melitensis* typing, thereby compromising their practicality and real-time monitoring efficiency. Whole-genome sequencing (WGS)-based analysis, such as single nucleotide polymorphism (SNP) analysis or core genome Multi-locus sequence typing (cgMLST), offer higher resolution and represent a promising alternative for enhanced genomic characterization and surveillance of this pathogen [10].

In recent years, the increasing accessibility of WGS has led to its adoption as the preferred method for in-depth analysis of *B. melitensis*, providing detailed insights into the genetic diversity within this species [11, 12]. Previous WGS studies conducted in various regions worldwide including countries in Asia [13, 14], North Europe [12, 15], and the Mediterranean region [16–18], have provided significant knowledge regarding the genomic characteristics of *B. melitensis*. However, there are currently no published studies on this topic in the Maghreb region, particularly in Tunisia.

In this study, we aimed to evaluate the genomic characteristics of *B. melitensis* strains isolated in south Tunisia using WGS, focusing on their clonal relationships, relatedness to international sequences, antimicrobial resistance determinants, and virulence factors over 35 years.

Results

General genomic characteristics

For the 24 *Brucella* sequences, the genomic analysis confirmed the identification of *Brucella melitensis.* The sequencing data indicates an average read count of 307,766 (min 183,708, max 440,079). Genome assembly yielded an average genome size of 3,286,965 bp (min 3,285,073 bp, max 3,293,649 bp) with an N50 average value of 170,579 bp. All sequences' total contig sequence lengths covered more than 99% compared to the reference *B. melitensis* 16 M genome. The annotation tool analysis showed that the protein-encoding genes ranged from 3,131 to 3,148. All isolates had three copies of rRNA, while the number of tRNA copies varied from 47 to 51 (Supplementary Table 2). The pangenome analysis of all sequences revealed a total of 3,304 genes. Among these, 3,054 core genes were shared by all isolates, in addition to 41 softcore genes, 73 shell genes, and 136 cloud genes.

The MLST-9 analysis assigned all Tunisian *B. melitensis* isolates to a single sequence type, ST11. The MLST-21 scheme identified two distinct ST profiles: ST89 ($n=18$) and ST114 ($n=6$). These two STs differed only on the *dll*A locus.

Based on 1,764 core genes, the cgMLST analysis indicated that 1,645 loci were detected in all strains with 1497 being identical. This analysis suggests that four profiles, cgST 680 ($n=9$), 677 ($n=5$), 610 ($n=4$), and 512 ($n=6$), were identified, with the number of allele differences among these profiles ranging from 3 to 158.

Phylogenetic analysis of 24 Tunisian isolates

The SNP analysis with the reference sequence revealed 3,247 cgSNP sites including 2,028 located on chromosome 1 and 1,219 on chromosome 2. The 24 *Brucella* genomes exhibited differences ranging from 2,944 to 3,036 SNPs compared to the reference sequence (*B.melitensis* 16 M). The pairwise comparison of core-genome SNPs between Tunisian isolates revealed 0 to 143 SNPs. The phylogenetic tree showed that 23 isolates were grouped into one cluster, with pairwise cgSNP difference between 0 and 77 (Fig. 1). The remaining singleton isolate (BR4) presented several pairwise SNPs from 107 to 143 with the other strains.

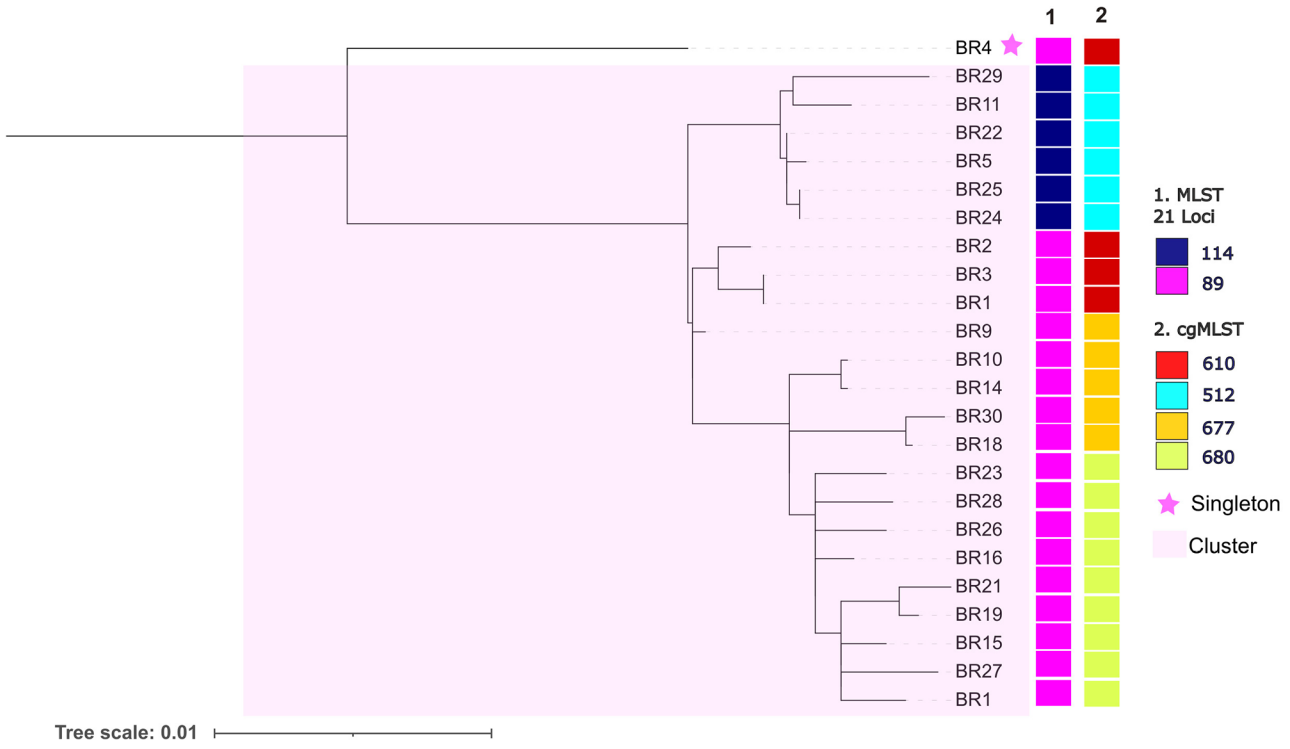

Fig. 1 Phylogenetic tree based on cgSNPs analysis of the 24 Tunisian *B. melitensis* sequences

Comparison with international sequences

The international Maximum Likelihood phylogenetic analysis identified a total of 6,971 core-genome SNP sites. The comparative analysis revealed the distinct topology of *B. melitensis* phylogeny, which consists of four major lineages: American, African, East Mediterranean, and West Mediterranean (WM). Comparison with international sequences showed that Tunisian strains are grouped within the WM clade. Within the WM lineage, the pairwise SNP differences ranged from 0 to 504 (Fig. 2). The WM lineage differed from the American lineage by 1,607 to 1,950 SNPs, from the African lineage by 1,643 to 1,925 SNPs, and from the East Mediterranean lineage by 1,534 to 1,856 SNPs.

Within the WM clade, isolates were grouped into two sub-clades (A and B). The sub-clade A included genomes from France, Italy, Sweden and the USA, with a pairwise number of SNP from 0 to 491. The sub-clade B grouped Tunisian strains along with genomes from Morocco, Algeria, Egypt, Eritrea, Italy, and the USA, with a pairwise number of SNP between 0 and 179. The pairwise SNP differences between sub-clades A and B ranged from 247 to 504 SNPs. Within sub-cluster B, strains from Tunisia, Morocco, Algeria, Egypt, Eritrea, Italy and the USA shared the same MLST-21 sequence types (ST89 and ST114).

The Tunisian cluster, consisting of 23 out of 24 Tunisian isolates, showed high genetic similarity with other publicly available Tunisian strains isolated from human cases, with pairwise SNP differences ranging from 0 to 19. All Tunisian strains, except BR4, were highly related to human strains from the Maghreb region, with pairwise SNP differences of 7 to 19 with Algerian isolates and 15 to 27 with Moroccan strains. Additionally, Tunisian strains were genetically similar to a human strain from Eritrea, with SNP differences ranging from 4 to 16. Slightly lower genetic relatedness was observed with human and animal Italian isolates (54 to 107 SNPs), the Egyptian human strain (94 to 106 SNPs), and one animal strain from the USA (67 to 79 SNPs).

Of note, the Tunisian singleton BR4 was more genetically distant to isolates from the Maghreb region and Eritrea compared to the Tunisian cluster, with a pairwise SNP difference of 71 SNPs with the Moroccan strain, 63 SNPs with the Algerian isolate, 60 SNPs with the Eritrean strain, and 56 to 68 SNPs with other Tunisian sequences.

Antimicrobial resistance determinants and virulence factors

The analysis of antimicrobial resistance (AMR) determinants using several databases revealed that no classical resistance genes were identified in the 24 Tunisian *B. melitensis* sequences. However, the presence of the multiple peptide factors (*Brucella_suis_mpr*F) protein and efflux-related genes *bep*C, *bep*D, *peb*E, *bep*F and *bep*G was noted.

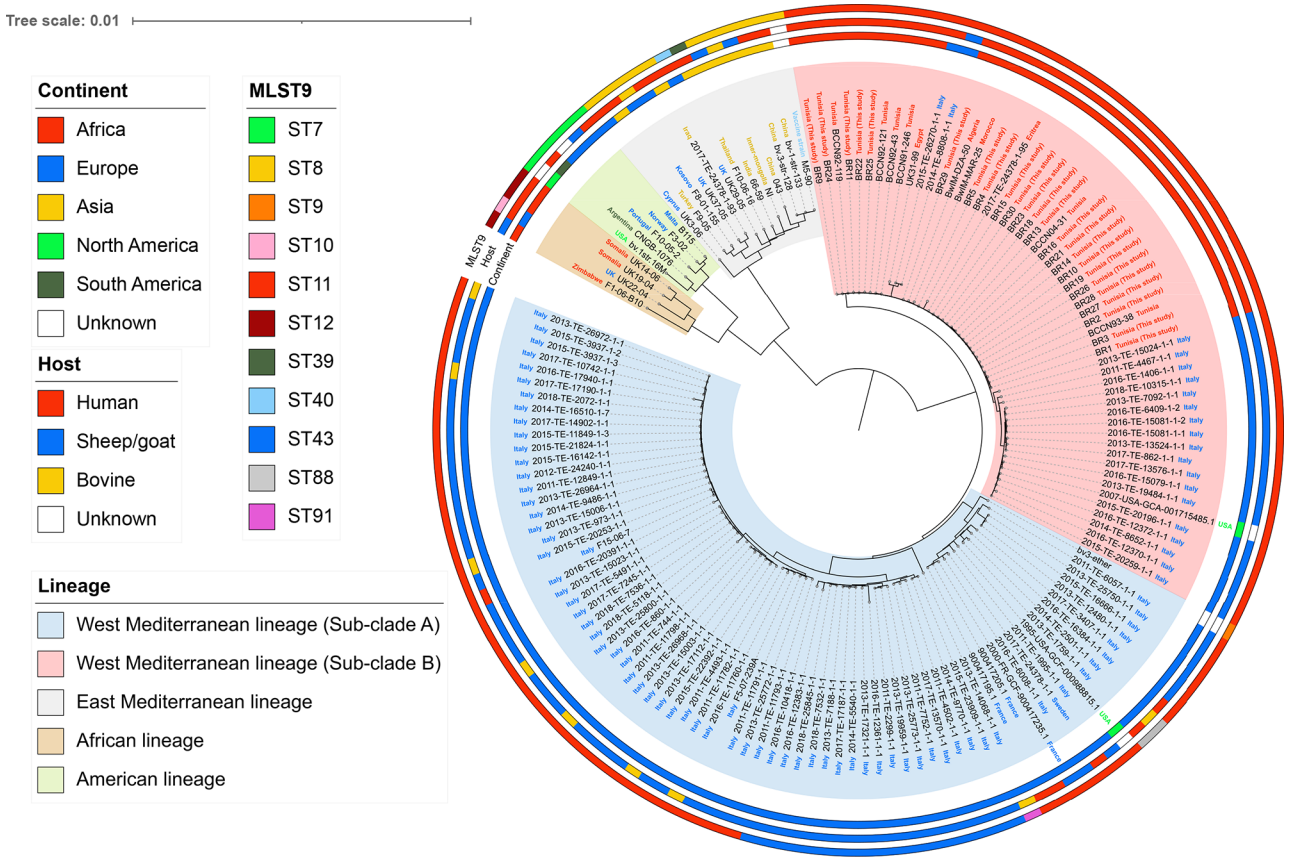

Fig. 2 Maximum-likelihood phylogenetic tree of Tunisian *B. melitensis* strains with international sequences based on cgSNPs analysis

Analysis of international sequences revealed that the *bep* genes were present in all strains, while *mpr*F was detected in 97% of the strains ($n = 148$) across all lineages (Supplementary Table 3).

In addition, no previously described AMR-associated SNPs were detected within the antibiotic resistance-associated genes *rpo*B, *fol*A, *fol*P, *gyr*A, *gyr*B, *par*C, *par*E. However, other SNPs were identified within these genes: G3747A (M1249I) in *rpo*B, C1795G (L599V) and C1557T(synonymous) in *gyr*A, G2188A (A730T) in *gyr*B and G298A (A100T) in *par*C.

In total, 67 virulence genes were identified in Tunisian *B. melitensis* isolates (Fig S1). These genes are mainly involved in the regulation and expression of the Type IV secretion system in *Brucella* ($n = 26$) and the immune modulation ($n = 35$), mainly through the synthesis, maturation, and functioning of lipopolysaccharide (LPS) ($n = 32$). Comparison with international sequences indicated that these genes were consistently present in over 98% of strains, except for *vir*B10 (88%; $n = 132$) and BPE043 (94%; $n = 141$). Notably, no differences in the distribution of these two virulence genes were observed across all lineages.

Discussion

Brucellosis remains endemic in many countries, with a significant burden on animals and humans. In Tunisia, *B. melitensis* is the primary causative agent of brucellosis [19]. Understanding the local epidemiology of this pathogen is crucial for both regional and international public health contexts, and molecular typing plays an essential role in this regard. In this approach, WGS is a powerful tool that enables the detailed characterization of genetic variations and the tracing of spreading pathways. This study utilized WGS to analyze Tunisian *B. melitensis* isolates, assess the relationships between strains circulating over 35 years, examine their relatedness with international sequences, and detect antimicrobial resistance genes and virulence factors.

Using WGS, all studied isolates were confirmed as *B. melitensis*, which is the most frequently isolated biovar among both humans and livestock in the Mediterranean region [20]. MLST-9 typing revealed that all examined isolates belonged to sequence type 11 (ST11). This sequence type has been previously documented in Mediterranean strains from Egypt, Morocco, Algeria, and Italy [15]. The analysis of MLST-21 and cgMLST loci showed closely related STs and cgMLST profiles which, along with the low pairwise number of SNPs, demonstrated

significant genetic stability of *B. melitensis* in Tunisia over more than three decades. Previous studies highlighted the genomic homogeneity of *B. melitensis*. Aman Ullah Khan et al. (2021) observed that Egyptian *B. melitensis* isolates recovered between 2014 and 2017, from both humans and animals, were closely related with a pairwise number of core genome SNP differences between 0 and 119 [18]. Additionally, a Greek study revealed highly similar cgMLST profiles within *B. melitensis* clusters over 10 years, evidenced by the low number of pairwise allelic differences (2 to 38 alleles) [17].

International phylogenetic analysis identified four main lineages of *B. melitensis* as reported by Janowicz et al. (2020). Our findings indicated that Tunisian *B. melitensis* isolates belonged to the WM lineage, also known as genotype I [11]. This lineage is recognized as the most basal form of *B. melitensis* and includes samples that date back 700 years [15]. Our study identified a close genetic relationship between Tunisian strains and isolates recovered from Morocco, Algeria, Egypt, and Italy compared to other Mediterranean countries such as Turkey, Cyprus and Malta. These WGS findings are consistent with previous studies using conventional techniques. Akar et al. (2022) conducted MLVA analysis on strains from various Mediterranean countries, including Egypt, Turkey, Italy, Portugal, Greece, and Tunisia. Their results revealed that the Tunisian *B. melitensis* strain isolated from sheep in 2017 clustered most closely with strains from Italy and Egypt [21].

Phylogenetic investigation indicated that Tunisian strains were more related to North African isolates than European ones. This close genetic relationship suggests regional patterns of transmission and evolution, likely influenced by historical trade and movement within the Mediterranean region. This observation was highlighted by Johansen et al. (2018), underscoring a notable correlation between travel history and genetic epidemiological sequence data [22]. However, it should be emphasized that the comparison was restricted to human strains from North Africa due to the unavailability of animal sequences. These restrictions may be attributed to the limited resources available for genomic surveillance in developing countries, as well as a comparatively lower focus on the epidemiology of animal infections relative to research on human brucellosis [12].

Tunisian strains also showed a high degree of genetic similarity to an isolate from a Swedish patient with a travel history to Eritrea (East Africa). Prior MLVA16 analyses indicated that the genetic profile aligns closely with those observed in strains from Tunisia [12]. The source of this strain remains unclear. It is uncertain whether it was endemic to Eritrea or introduced through the trade of infected animals or contaminated milk products with Maghreb countries (Libya, Tunisia, Algeria, Morocco). Further genomic investigations of *B. melitensis* in Eritrea and throughout Africa are imperative to enhance our understanding of the pathways through which the disease spreads across the continent.

Of note, the Tunisian cluster was more genetically similar to Morocco and Algeria strains than the Tunisian singleton BR4. However, this singleton strain demonstrated a closer genetic affinity to the Tunisian cluster than the Egyptian strain. This finding suggests that BR4 may have been imported from another North African country. However, due to the unavailability of epidemiological data regarding this case when the strain was first isolated in 1990, the source of BR4 remains unknown.

Due to the lack of guidelines regarding AMR assessment of *Brucella* spp. from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) as of 2024, our current study did not assess the phenotypic resistance profile. Therefore, WGS offers an alternative solution to address this issue, enabling the exploration of genetic markers that could predict resistance. WGS analysis revealed the absence of classical antimicrobial genetic determinants in this study, suggesting the susceptibility of our strains to most antibiotics.

In particular, none of the SNPs identified within *rpo*B, *gyr*A, *gyr*B and *par*C genes were previously associated with resistance. Specifically, the G3747A mutation within the *rpo*B gene, which is a commonly used locus for genotyping *B. melitensis*, has been previously proposed as a molecular marker for biovar 3 [23] without being involved in rifampicin resistance [24]. Similarly, *gyr*A mutations are also present in the biovar 3 reference sequence, suggesting they represent natural polymorphisms not linked to resistance. The identified mutations in *gyr*B and *par*C have not been previously described to confer resistance in *B. melitensis.* However, further research, including phenotypic assays and functional analyses, is needed to confirm the implications of these mutations.

In addition, all Tunisian isolates harboured the *Brucella suis-mpr*F and *bep*CDEFG genes. The *mpr*F gene has been reported to affect susceptibility to cationic antibiotics such as daptomycin, gentamicin, moenomycin, and vancomycin in *Staphylococcus aureus* [25, 26], whereas *bep* genes were reported to increase resistance to certain antibiotic compounds, such as tetracycline, doxycycline, chloramphenicol, and ciprofloxacin, in *B. suis* [27]. However, the presence of *mpr*F and *bep*CDEFG genes in *B. melitensis* has not been associated with phenotypic resistance in prior studies [28, 29]. The exact role of these genes in *B. melitensis* remains unclear and calls for further investigation.

Comparative analysis revealed that international *B. melitensis* strains across all lineages shared these genes. Our results align with previous studies indicating that

the *Brucella* genome remains highly conserved and lacks plasmids, with no observed evidence of genetic material transfer [30]. These characteristics suggest a relatively low risk of acquiring antibiotic resistance. Previous studies from Egypt [29], Turkey [13], Iran [28], China [31], and Norway [22] indicated a consistent susceptibility profile to tetracycline, doxycycline, streptomycin, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole. In contrast, research on isolates collected in China revealed resistance to azithromycin, suggesting a potential emerging trend of *Brucella* developing resistance to this antibiotic [30]. Moreover, previous studies have reported probable resistance to rifampin in cases of brucellosis. These reports originate from various countries, including Egypt (64%) [32], Iran (35%) [33], and Qatar (48%) [34]. This resistance may be attributed to the similarity in treatment protocols used for both brucellosis and tuberculosis in the Middle East region [14].

Regarding the virulome of *B. melitensis* isolates, 67 genes were consistently detected in all Tunisian strains. Most of these genes are involved in two critical processes: the production of lipopolysaccharide (LPS) and the synthesis of the type IV secretion system (T4SS). LPS is essential for maintaining the structural integrity of the cell envelope of *B. melitensis* [35], shielding it from complement-mediated bactericidal effects and antimicrobial peptides encountered during interactions with host phagocytes [36]. LPS also serves roles in adhesion to mammalian cells and immune evasion, facilitated by its low endotoxin activity that prevents recognition by Toll-like receptor 4 (TLR4) [37, 38]. This evasion strategy includes inducing premature cell death in human neutrophils infected with *Brucella*, promoting uptake by macrophages and dendritic cells without triggering an inflammatory response [39].

On the other hand, the T4SS plays a pivotal role by modulating the host immune response and controlling the intracellular trafficking of *Brucella*-containing vacuoles within host macrophages [40]. This ability helps *B. melitensis* evade phagolysosome degradation, ensuring its survival within the host cell [39].

These findings are concordant with what has been previously documented about *Brucella* virulence. *Brucella* is a facultative intracellular bacteria that is known to lack virulence plasmids, and its pathogenicity is mainly linked to its ability to evade the host immune reaction, rather than with the synthesis of proteases, exotoxins, fimbria, capsules, pili, and cytolysins [41]. Most virulence factors identified in this study were previously described in other Mediterranean and Asian *B. melitensis* isolates, including those from Egypt [18, 42], Italy [16], and Iran [28]. Furthermore, our comparative analysis demonstrated that these genes were consistently present in international strains, with no notable difference in their distribution across all lineages. This finding suggests a shared repertoire of virulence genes among *B. melitensis* strains, underscoring the stability of the *Brucella* genome.

Overall, this study reports the first genomic investigation of *B. melitensis* strains circulating in Tunisia. Our findings revealed a significant genetic stability over three decades, a consistent susceptibility profile to major antibiotics, and a standard set of virulence factors shared with other Mediterranean strains. These results highlight the importance of regional surveillance and international collaboration in managing and controlling brucellosis. Further molecular investigations are needed to fully understand the genetic mechanisms underlying antimicrobial resistance and virulence in *Brucella* species.

Materials and methods

Brucella melitensis isolates

Between 1988 and 2022, 88 *B. melitensis* isolates were recovered from humans in the laboratory of microbiology - Habib Bourguiba University Hospital of Sfax-Tunisia. This hospital is the primary medical centre for an urban population of one million residents and serves as a referral centre for a larger population from southern Tunisia. From these isolates, 24 were randomly selected for whole-genome sequencing. These strains were isolated from blood ($n = 21$), abscesses ($n = 2$), and a cardiac valve ($n = 1$). Among the 24 strains, eight were classified as biovar 3 through a phenotypic analysis conducted by the National Research Institute for Agriculture, Food and Environment (INRA) in France.

Whole-genome sequencing and quality assessment

Following biological security rules, total genomic DNA from the 24 selected *B. melitensis* samples was extracted using the QIAamp DNeasy blood and tissue method (Qiagen Inc., Valencia, CA). WGS was conducted on the Illumina NextSeq 500 or NovaSeq platform using 2×150 bp paired-ends. The quality assessment of the paired-end Illumina sequence data was performed using FASTQC (Version 0.11.9) [43]. Adaptors and low-quality reads were removed from the dataset using Trimmomatic Version 0.39 [44]. The integrity of the data was evaluated utilizing Kraken2 (Version 2.1.3) [45] to check for any potential sequence contamination. *Brucella melitensis* strain 16 M (Genbank accessions NC_003317 and NC_003318) was used as the reference genome for subsequent analyses. Theoretical coverage was determined by aligning the reads from each strain to the reference genome using the Burrow-Wheeler Aligner (v. 0.7.17) [46] and SAMtools (v.1.16) [47] to generate a BAM file. The SAMtools depth function was employed to compute the average read coverage. The assembly was performed using Shovill (v 1.1.0) with default parameters [48]. The assembled genomes were analyzed with QUAST (v 5.0.2)

[49] to evaluate the number of contigs, the N50 score, and the total length. Finally, the genome annotation process was performed using Prokka (Version 1.11) [50].

WGS-based genotyping

Mash (Version 2.1) was used for species confirmation. Multi-locus sequence type (MLST) profiles for the *Brucella* isolates were determined using pubMLST (https://pubmlst.org/). Two typing schemes were employed, one with 9 loci (*gap*, *aroA*, *glk*, *dnaK*, *gyrB*, *trpE*, *cobQ*, *int_hyp*, *& omp25*) and the other with 21 loci (*gap*, *aroA*, *glk*, *dnaK*, *gyrB*, *trpE*, *cobQ*, *int_hyp*, *omp25*, *prpE*, *caiA*, *csdB*, *soxA*, *leuA*, *mviM*, *fumC*, *fbaA*, *ddlA*, *putA*, *mutL*, *&acnA*). Additionally, *B. melitensis* sequences were analyzed using cgMLST with the PubMLST platform which is based on 1,764 core genes [51].

Core-genome SNPs (cgSNPs) were identified using Snippy (Version 4.6.0, available at https://github.com/tseemann/snippy), and the construction of the maximum likelihood tree for the core genome was accomplished using IQ-TREE [52]. Visualization of cgSNPs phylogenetic trees was achieved using iTOL (Version 4, https://itol.embl.de/). The SNP distance between each pair of genomes was computed using pairsnp (https://github.com/gtonkinhill/pairsnp). For SNP annotation, the SnpEff tool implemented in Snippy was employed to predict the coding effects of cgSNPs and assign cgSNP variants of the isolates to their respective positions on the two chromosomes [53].

Comparison with international sequences

A total of 128 publicly available *B. melitensis* sequences, originating from 24 different countries were downloaded from NCBI genomes (supplementary Table 1). These sequences were selected based on the classification of Janowicz et al. (2020) and Tan et al. (2015) as well as other publicly available genomes from North Africa [11, 16]. The relationship of studied strains with international sequences was evaluated by determining pairwise SNP differences and the construction of maximum likelihood tree, as described above.

Antimicrobial resistance determinants and virulence factors detection

The assembled genomes were screened for antimicrobial resistance genes through several databases, including the Resfinder database [54], the Comprehensive Antibiotic Resistance Database (CARD) [55], and the NCBI AMRFinder Plus [56]. Additionally, amino acid substitutions associated with resistance within the *rpo*B, *fol*A, *fol*P, *gyr*A, *gyr*B, *par*C, and *par*E genes were detected using snpEff Version 4.3.1, as implemented in Snippy, which also predicts effects on the coded gene product [53]. The virulence finder database (VFDB) through Abricate (https://github.com/tseemann/abricate) determined the potential virulence genes [57].

Abbreviations

cgMLST	Core genome multi-locus sequence typing
LPS	Lipopolysaccharide
MLST	Multi-locus Sequence Typing
MLVA	Multi-locus variable-number tandem repeat analysis
SNP	Single nucleotide polymorphism
ST	Sequence Type
WGS	Whole-genome sequencing
WM	West Mediterranean

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03802-1.

Supplementary Material 1

Supplementary Material 2

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Author contributions

Conceptualization, BK, FS, NBA and AH; investigation, NBA, MG, SM, SK, FM and AH; Formal analysis, BK; Visualization: BK and FS, writing—original draft preparation, BK and FS; writing—review and editing, FS, NBA and AH, supervision, MBJ, HK and AH. All authors have read and approved the manuscript for publication.

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Data availability

Sequencing data were uploaded to NCBI under BioProject PRJNA1127036, and the metadata for all the isolates sequenced in this study are listed in Supplementary Table 2.

Declarations

Ethics approval and consent to participate

This study was approved by the institutional Ethics Committee of the Faculty of Medicine of Sfax-Tunisia (Decision N°5/2024), and the committee waived the consent to participate in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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