Original Article	*Curr. Appl. Sci.*, **2025**, *3*(1):11-20 DOI:10.22034/cas.2023.391375.1031	Current Applied Sciences

Molecular Identification of *Brucella* Bacteria Using *BLS* and *Omp31* Genes

Masoumeh Noura[1], Hossein Kamaladini[1]*, Fatemeh Haddadi[1], Mohsen Najimi[2]

[1] *Department of Biology, Faculty of Sciences, University of Zabol, Zabol, Iran*

[2] *Department of Pathobiology, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran*

Corresponding author's e-mails: *h.kamaladini@uoz.ac.ir* and *hu_kamaladiny@yahoo.com.au*

Article Information

Received: 30 March 2024
Revised: 18 May 2024
Accepted: 19 May 2024
Published online: 02 February 2025

Keywords

Brucella
Brucellosis
BLS gene
Omp31 gene
Multiplex polymerase chain reaction
Polymerase chain reaction

Abstract

Brucellosis or Malta fever (Mediterranean fever) is an important zoonosis caused by different species of *Brucella* – a small, Gram-negative, aerobic, non-motile, non-encapsulated, and non-spore-forming coccobacillus. Brucellosis can be easily transmitted to humans by *Brucella*-contaminated blood, meat, or milk. The lack of an effective tool for vaccination or efficient treatment has necessitated rapid bacterial detection methods for preventing this disease. In this study, we optimized the molecular detection of *Brucella* through polymerase chain reaction (PCR) and multiplex-PCR. To this end, the *Omp31* and *BLS* genes were amplified, resulting in two fragments of 347 bp and 256 bp, respectively. PCR and multiplex-PCR specificity and sensitivity for genomic DNA were 100% and 0.39 ng/µL, respectively. The detection time of *Brucella* was less than 2 hours, which is obviously shorter than the identification time of the traditional methods like culture, which usually takes more than a day. Given the high specificity and sensitivity of *Brucella* detection with these genes through multiplex-PCR, we suggest this approach for evaluating the contamination of livestock in veterinary reference laboratories.

1. Introduction

Brucellosis is one of the most common zoonotic diseases, which affects more than 500,000 people worldwide every year and is classified as a forgotten neglected zoonosis as per the world health organization (WHO) [1, 2]. Brucellosis is transmitted by exposure to contaminated animals or animal products [3] and could also be transmitted through the inhalation of aerosols and contact with secretions [1]. This disease is caused by the *Brucella* bacterium - an intracellular parasite and a small, Gram-negative, aerobic, non-motile, non-encapsulated, and non-spore-forming coccobacillus. Various strains of *Brucella* with different pathogeneses and hosts have been recognized according to the host type and antigenic changes [4]. *Brucella melitensis* is a sheep- and goat-

contaminating species and the most significant human-affecting strain that can infect even camels and cows [5]. Brucellosis has economic and health impacts since it can result in abortion, reduced milk production, infertility of the affected livestock, and human infection [6].

Brucellosis manifests with fever, arthralgia, and fatigue in humans [7]. Five occupational groups are exposed to this disease: ranchers, slaughterhouse workers, veterinarians, laboratory employers, and hunters [8]. Eliminating the disease pathogen by boiling or pasteurizing milk and its products is the most important way of brucellosis prevention [9]. In addition, avoiding contact with secretions and tissues of contaminated animals, such as blood and livestock abortion secretions, [10] and informing the at-risk groups about the transmission ways and prevention methods [11] can help reduce the occurrence of this disease.

The lack of an effective control program in animals is the main cause of brucellosis spread in the world [12]. Antibiotics are applied to help control the infection, and for brucellosis most often used antibiotics are minocycline or doxycycline, enrofloxacin, and rifampin. However, no treatment is completely effective at eliminating the bacteria, as it can persist in tissues. Many studies have been performed to develop fast detection and diagnosis systems to overcome the rising tide of antibiotic resistance over the last several years [13] since rapid, cost-effective bacterial detection methods can help control the disease's spread and transmission [14].

The traditional isolation and culture methods remain the diagnostic gold standard for many bacterial infections like *Brucella*. However, the results are not decisive and valid; in addition, some phenotype-evaluating tests are time-consuming [15]. On the other hand, the close genetic relationship of different species in the *Brucella* genus impedes intra-species identification by traditional methods [16]. Moreover, bacterial culture-based methods are associated with the risk of contamination of laboratory staff; therefore, molecular methods are used for detecting infectious diseases [17]. Different strategies have been applied to detect disease-causing agents, such as real-time quantitative polymerase chain reaction (qPCR), enzyme linked immunosorbent assay (ELISA), fluorescence assay using aptamers, and various biosensing approaches, including immunosensor, biosensors and nanobiosensors using gold nanoparticles, silver nanoparticles, carbon nanotubes, and magnetic nanoparticles [13, 18]. Despite many advantages of these techniques cost of the detection and availability of facilities are the limitations of using them for the early detection of pathogens. Among molecular techniques for detection of zoonotic pathogens, conventional strategies like single/multiplex PCR have been widely applied, and these techniques provide efficient differentiation of species [19]. Amplification of unique gene sequences of an organism using PCR enables the analysis of sensitivity at which organisms can be analyzed [13]. In multiplex-PCR, more than one gene sequence are simultaneously amplified in a single reaction, increasing the accuracy and reducing the costs [19, 20]. Genes that are responsible for making outer membrane proteins have been used for molecular identification of *Brucella*. A few of the more important genes are *Omp2*, *Omp31*, *Omp28*, *IS711*, *16S rRNA*, and *bcsp31* gene [21].

The rapid and sensitive detection of pathogenic bacteria, especially zoonotic pathogens, is crucial for ensuring food safety and public health [18] by minimizing the dosage and resistance of antibiotic modalities [13]. Hence in this study, we optimized PCR and multiplex-PCR for the molecular detection of *Brucella*, an important zoonotic bacterium, using the *Omp31* and *BLS* genes.

2. Materials and Methods

2.1 *Samples and primers*

The *Brucella* bacterial DNA was obtained from the Veterinary School archive of University of Zabol and used the *BLS* and *Omp31* genes' sequences (NCBI) for designing the primers for PCR and multiplex-PCR. The primers were designed using primer3 plus software and the properties of the designed primers were evaluated by primer BLAST site and oligo analyzer tools of Integrated DNA Technology (IDT). The primers were F: 5'CAAAGCTGTCCGAACAAGAC3' and R: 5'ATGACGATAGATGCCGCCG3' for *BLS*, resulting in a 256 bp fragment and F: 5'TTGACACCTTCTCGTGGACC3' and R: 5'AACCATGAGGCGTTCGGTAG3' for *Omp31* resulting in a 347 bp fragment.

2.2 *PCR and multiplex PCR*

Brucella was identified using *BLS* and *Omp31* genes separately through PCR and together through multiplex-PCR. First, the PCR gradient was performed to obtain the optimal temperatures for the designed pair primers using the primers of each gene individually and both genes collectively. The reaction mixture consisted of 2X red Master Mix (Amplicon, Denmark), 0.2mM of each forward and reverse primer, 50 ng/µL template DNA, and deionized water in a total volume of 25 µL. The microtubes were put in the thermal cycler (Eppendorf, Germany) and evaluated at 57.5, 58.5, 59.5, 60.5, and 61.5 °C to confirm and optimize the annealing temperature of the primers. The PCR program consisted of the initial denaturation at 94 °C for 5 minutes, 30 cycles of denaturation at 94 °C for 30 s, annealing at 56.5-62.5 °C for 35 s, and extension at 72 °C for 30 s, and final extension at 72 °C for 10 min. The PCR products were electrophoresed on 1.5% agarose gel using TBE 1X buffer, stained with safe stain, and analyzed in a gel documentation system (Cambridge, France).

2.3 *Specificity and sensitivity of multiplex PCR*

To assess the specificity of the designed primers, multiplex-PCR was performed using the DNA of *Brucella* and the DNA of Staphylococcus aureus, Streptococcus, and Escherichia coli as negative controls. Sensitivity was evaluated through serial dilution of *Brucella* DNA, with an initial concentration of 50 ng/µL, by adding a certain amount of distilled water and preparing the dilutions 25 ng/µL, 12.5 ng/µL, 6.25 ng/µL, 3.12 ng/µL, 1.56 ng/µL, 0.78 ng/µL, and 0.39 ng/µL.

2.4 *The optimized method efficiency*

After optimization of multiplex-PCR, we evaluated 40 blood samples of sheep collected from villages of Zabol, Iran, the contamination of which with *Brucella* has been confirmed through the serological test in the Zabol Veterinary Office lab. The infected blood's DNA was extracted using the Blood DNA Extraction Kit (ZandBiotech Co., Iran).

3. Results and Discussion

3.1 *Specificity of Omp31 and BLS genes in Brucella*

The specificity of *Omp31* and *BLS* genes of *Brucella* was evaluated using the basic local aliment search tool (BLAST) platform in National Center for Biotechnology Information (NCBI). The results showed a 100% similarity in different strains of *Brucella*, with no similarity to other microorganisms.

3.2 *PCR*

The temperature gradient, performed to confirm and optimize the annealing temperature, showed that 256 bp and 347 bp fragments of *Omp31* and *BLS* genes were amplified at 57.5, 58.5, 59.5, 60.5, and 61.5 °C. All studied

temperatures are appropriate for the amplification of these genes; however, the band formed at 57.5 °C had a good quality and resolution for both genes and hence was used as the optimum temperature of primers for evaluating the specificity and sensitivity of PCR for detecting *Brucella* using the mentioned genes (Figure 1A and 1B).

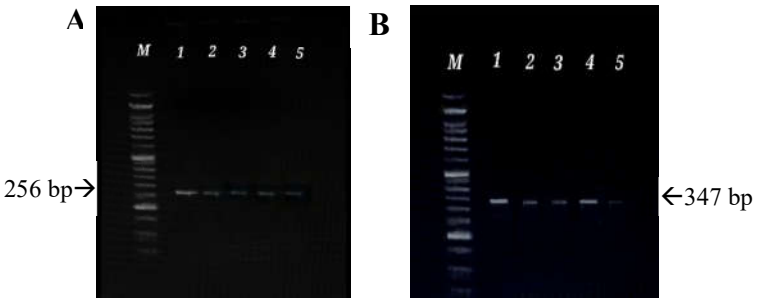

Figure 1. Temperature gradient of PCR for annealing the designed primers of *Omp31* and *BLS* genes; M: gene marker, 50 bp. A) The *BLS* gene temperatures; 1: 57.5 °C, 2: 58.5 °C, 3: 59.5 °C, 4: 60.5 °C, 5: 61.5 °C. A) The *Omp31* gene temperatures; 1: 57.5 °C, 2: 58.5 °C, 3: 59.5 °C, 4: 60.5 °C, 5: 61.5 °C

3.3 *Specificity and sensitivity of PCR using the BLS gene*

Amplification of the 256 bp fragment in the *Brucella* genomic DNA-containing reaction mixture and the lack of negative control bands (*S. aureus*, *Staphylococcus*, and *E. coli*) indicates the specificity of the primers designed for the *BLS* gene (Figure 2A). The sensitivity of PCR using the *BLS* gene primers showed that the gene fragments were amplified at concentrations 50 ng/µL, 25 ng/µL, 12.5 ng/µL, 6.25 ng/µL, 3.12 ng/µL, 1.56 ng/µL, and 0.78 ng/µL. The band at 0.78 ng/µL was weak but visible, and no amplification occurred at 0.39 ng/µL (Figure 2B).

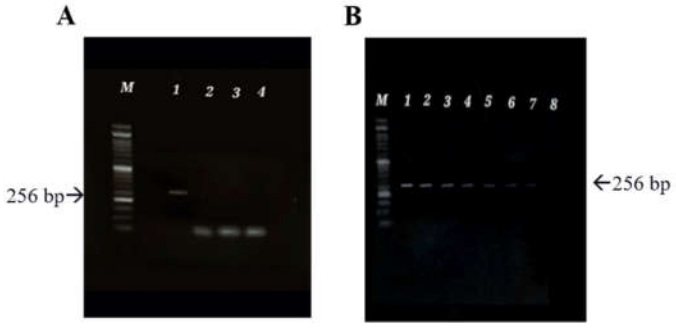

Figure 2. A) PCR specificity of the *BLS* gene primers, M: gene marker, 50 bp; 1: *Brucella*, 2: *S. aureus*, 3: *Staphylococcus*, 4: *E. coli.* B) PCR sensitivity of the *BLS* gene primers through genomic DNA dilution method; M: gene marker, 50 bp; 1:50 ng/µL, 2: 25 ng/µL, 3: 12.5 ng/µL, 4: 6.25 ng/µL, 5: 3.12 ng/µL, 6: 1.56 ng/µL, 7: 0.78 ng/µL, 8: 0.39 ng/µL

3.4 *Specificity and sensitivity of PCR using the Omp31 gene*

Amplification of the 347 bp fragment in the *Brucella* genomic DNA-containing reaction mixture and the lack of negative control bands (*S. aureus*, *Staphylococcus*, and *E. coli*) indicates the specificity of the primers designed for the *Omp31* gene (Figure 3A). The sensitivity of PCR using the *Omp31* gene primers showed that the gene fragments were amplified at concentrations 50 ng/µL, 25 ng/µL, 12.5 ng/µL, 6.25 ng/µL, 3.12 ng/µL, 1.56 ng/µL, and 0.78 ng/µL. However, no amplification occurred at 0.39 ng/µL (Figure 3B).

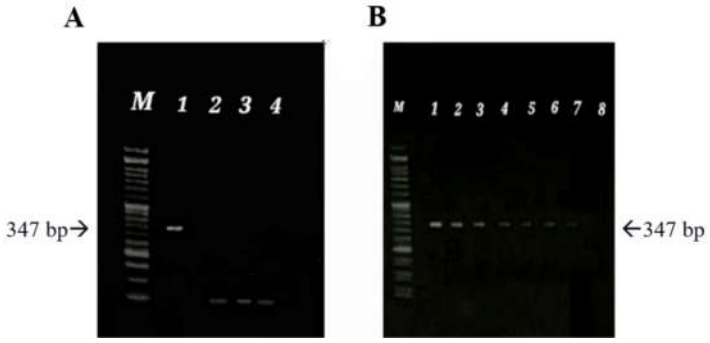

Figure 3. A) PCR specificity of the *Omp31* gene primers, M: gene marker, 50 bp; 1: *Brucella*, 2: *S. aureus*, 3: *Staphylococcus*, 4: *E. coli*. B) PCR sensitivity of the *Omp31* gene primers through genomic DNA dilution method; M: gene marker, 50 bp; 1:50 ng/µL, 2: 25 ng/µL, 3: 12.5 ng/µL, 4: 6.25 ng/µL, 5: 3.12 ng/µL, 6: 1.56 ng/µL, 7: 0.78 ng/µL, 8: 0.39 ng/µL

3.5 *Multiplex PCR*

Temperature gradient, performed to confirm and optimize the annealing temperature of multiplex-PCR, showed that 256 bp and 347 bp fragments of *Omp31* and *BLS* genes were amplified at 57.5, 58.5, 59.5, 60.5, and 61.5 °C. Given that the band formed at 57.5 °C had a good quality and resolution for both genes, it was used as the optimum temperature of multiplex-PCR (Figure 4).

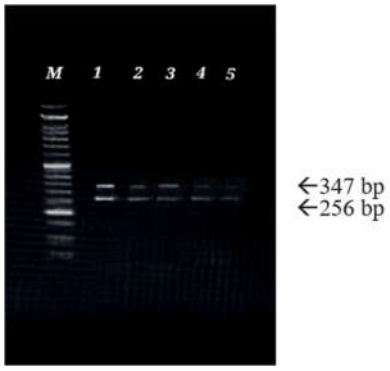

Figure 4. Temperature gradient of PCR for annealing the designed primers of *Omp31* and *BLS* genes; M: gene marker, 50 bp; 1: 57.5 °C, 2: 58.5 °C, 3: 59.5 °C, 4: 60.5 °C, 5: 61.5 °C

3.6 *Specificity and sensitivity of multiplex PCR*

Amplification of the 256 bp and 347 bp fragments in the presence of *Brucella* genomic DNA and the lack of negative control bands indicated the specificity of the primers designed for the *BLS* and *Omp31* genes of *Brucella*.

The DNA of *Brucella* and that of *S. aureus*, *Staphylococcus*, and *E. coli* was evaluated through multiplex-PCR. The intended fragments were amplified only in samples containing *Brucella* genomic DNA. The lack of band formation in the negative control bacteria showed the specificity of the designed primers (Figure 5A). We determined the sensitivity of multiplex-PCR using the *Omp31* and *BLS* genes' primers and eight concentrations of *Brucella* DNA, resulting in 256 bp and 347 bp fragments at concentrations 25 ng/µL, 12.5 ng/µL, 6.25 ng/µL, 3.12 ng/µL, 1.56 ng/µL, and 0.78 ng/µL. Although these fragments were present at 0.78 ng/µL, the corresponding bands were weak compared to other dilutions. No amplification occurred at 0.39 ng/µL (Figure 5B). The multiplex-PCR sensitivity was 0.78 ng/µL.

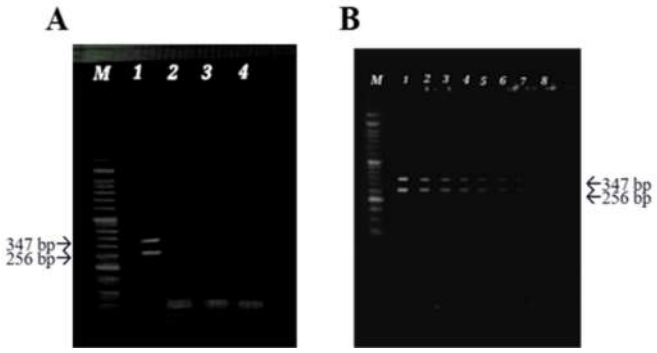

Figure 5. A) Specificity of the *Omp31* and *BLS* genes' primers in multiplex-PCR, M: gene marker, 50 bp; 1: *Brucella*, 2: *E. coli*, 3: *S. aureus*, 4: *Staphylococcus*. B) Multiplex-PCR sensitivity identified through genomic DNA dilution method; M: gene marker, 50 bp; 1:50 ng/µL, 2: 25 ng/µL, 3: 12.5 ng/µL, 4: 6.25 ng/µL, 5: 3.12 ng/µL, 6: 1.56 ng/µL, 7: 0.78 ng/µL, 8: 0.39 ng/µL

Evaluation of 40 *Brucella*-positive samples, confirmed by the serological tests, through multiplex-PCR showed that the 256 bp and 347 bp fragments were amplified in 35 samples of DNA extracted from blood.

This study aimed to detect *Brucella* infection through PCR and multiplex PCR targeting *Omp31* and *BLS* gene sequences. Outer membrane proteins (OMPs) are located on the bacterial surface and interact with host cells and immune response factors, whereby they can be important virulence factors of *Brucella* species. The *Omp31* gene is specific to *B. melitensis* species and encodes a 31kD outer membrane protein and antigen that is important for survival and proliferation by inhibiting apoptosis through TNF-α signaling following *Brucella* infection. The *BLS* is a protein with high immunogenicity and antigenic properties and can accept foreign proteins at its N-terminal end.

Brucella, the intracellular Gram-negative coccobacillus, can affect various wild and domestic animals [22]. This genus currently has at least 12 known species [23], including the four main species of *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis* [24]. The classic methods and new molecular approaches have confirmed the frequency of Brucellosis in Iran and reflect the expansion of various biovars of *B. melitensis* and *B. abortus* [25]. *B. melitensis* is the common cause of brucellosis in sheep and goats [26]. Although minocycline or doxycycline, possibly enrofloxacin antibiotics, can be used to control the infection, no treatment could be completely effective at eliminating the bacteria, as it can persist in tissues. Hence rapid and precise detection of *Brucella* is crucial for diagnosis, prevention, and better control of brucellosis [27].

In the present study, we evaluated the identification of *Brucella*, obtained from the reference laboratory, and results indicated sensitivity and specificity of 100% in detecting using multiplex-PCR for the genes *Omp31* and *BLS*. We assessed the efficiency of this method using 40 blood samples and showed a sensitivity of 100% for the two PCR-based methods used in this study.

The detection time of *Brucella* was decreased to less than 2 hours by direct use of livestock blood samples with no need for culture for molecular detection. According to the results, the genes *Omp31* and *BLS* individually can be used for detecting *Brucella*; however, simultaneous use of these specific genes can identify the infected samples more precisely and decisively. The *Omp31* gene is present only in *B. melitensis* [28]; as a result, the simultaneous use of this gene and other *Brucella*-specific genes helps detect the *B. melitensis* species with high specificity.

Various studies tried to detect *Brucella* in different samples by techniques such as ELISA and complement fixation test (CFT) [29], uniplex and multiplex PCR [19], nested PCR, in-house PCR, PCR-enzyme immunoassay [30, 31].

Abdulkaliq *et al*. investigated the PCR to identify the colonies isolated from the biochemical tests of *B. melitensis* isolates using primers that target a specific region of the genome (*IS711*). Also, multiplex PCR was used to determine four genes related to the virulence of *lps B, mgtA, Omp25,* and *CBG* in all positive samples [32].

Hosseinidoust *et al.* identified *B. abortus* with PCR and compared it with the culture method. They used primers for amplifying the gene segment *IS711* using the standard *Brucella* strains and showed that culture and serological methods have limited capability for differentiating *Brucella* species. Out of 42 serologically positive samples, the bacterium was grown in only six samples; and the sensitivity of culture and PCR methods was 40% and 60%, respectively, in livestock samples evaluated through culture and PCR. In addition, five human samples were serologically positive and grown in the culture medium, although only four samples were detected through PCR [33].

To understand the epidemiologic status of brucellosis in Iran, Dadar *et al.* (2019) molecularly detected *Brucella* species and biovars causing infection in animals and humans. All isolates pertained to *B. abortus* and *B. melitensis* species. Infection in sheep seems to be exclusively caused by *B. melitensis*, but the species *B. abortus* and *B. melitensis* were common among cows. The results showed that *B. melitensis* and *B. abortus* are the most common biovars in Iran [34].

Shafei *et al.* (2012) detected *B. abortus* and *B. melitensis* in the milk of cows and sheep in Kurdistan Province, Iran. They used PCR to show milk contamination and the non-preferred hosts of *B. abortus* and *B. melitensis* in cows and sheep. To this end, they collected the milk of 60 cows and 50 sheep with possible brucellosis from Kamyaran, Marivan, and Sanandaj cities, Iran. They extracted DNA directly from milk and performed PCR using primers designed for detecting the *Brucella* genus with primers of *B. abortus*, *B. melitensis*, and *IS711* for detecting *B. abortus* and *B. melitensis* species biovars. PCR showed that the milk of 20 cows and 22 sheep were positive for *the Brucella* genus. Out of 20 positive cow samples, 9 were *B. abortus,* and 2 were *B. melitensis*, and of 22 positive sheep samples, 15 were *B. melitensis,* and 1 was *B. abortus*. Since two cows were infected with *B. melitensis* and one sheep was infected with *B. abortus*, in herds in close contact, livestock can be affected by non-preferred species. According to the results, besides culture, it was suggested to use specific primers for detecting *B. abortus* and *B. melitensis* biovars for diagnosing brucellosis with PCR [35]. Aliyev *et al.* (2022) studied the molecular properties of *B. abortus* and *B. melitensis* isolated from cow milk in Azerbaijan, aiming to isolate and identify *Brucella* species. RT-PCR confirmed that the *B. abortus* and *B. melitensis* strains pertained to *the Brucella* genus. These researchers showed that *B. melitensis* has transmitted from small ruminants to cows, and cows should be considered the potential sources of *B. abortus* and *B. melitensis* for humans in the Ganja region [36].

4. Conclusion

Brucellosis is one of the major zoonotic diseases of public health importance. In this study, out of 40 clinical samples serologically positive for *Brucella*, PCR, and multiplex-PCR approaches using *Omp31* and *BLS* gene sequences showed 35 positive and five negative samples, indicating low sensitivity and accuracy of the

serological methods and emphasizing the importance of molecular methods for *Brucella* bacteria detection. Consequently, multiplex-PCR is a cost-effective and easy-to-use method in terms of enzyme and ingredients consumed, and high accuracy in a single reaction in which a few gene sequences are amplified simultaneously. It could be recommended to use the data of this study for further identification of *Brucella* in clinical samples.

Conflicts of Interest

The authors declare no conflict of interest associated with this manuscript.

References

1. Rahman MT, Sobur MA, Islam MS, Ievy S, Hossain MJ, El Zowalaty ME, Rahman AT, Ashour HM. Zoonotic Diseases: Etiology, Impact, and Control. *Microorg*., 2020, 8(9):1405.
2. Brambila-Tapia, AJL, Armenta-Medina D, Rivera-Gomez N, Perez-Rueda E. Main Functions and Taxonomic Distribution of Virulence Genes in *Brucella melitensis 16 M*. *PLoS ONE*, 2014, 9(6):e100349.
3. Middlebrook EA, Romero AT, Bett B, Nthiwa D, Oyola SO, Fair JM, Bartlow AW. Identification and distribution of pathogens coinfecting with *Brucella spp*., Coxiella burnetii and Rift Valley fever virus in humans, livestock and wildlife. *Zoonoses Pub. Health*, 2022, 69(3):175-194.
4. Mahon CR. Bacterial cell structure, physiology, metabolism, and genetics. In: Textbook of Diagnostic Microbiology. 2022, Philadelphia: Saunders, p 1.
5. Nicoletti PL. Relationship between animal and human disease. In: Brucellosis*:* Clinical and Laboratory Aspects*.* 2020, Boca Raton: CRC Press, pp. 41-51.
6. Ukwueze CS, Kalu E, Odirichukwu EO, Ikpegbu E, Luka PD. Overview of human and animal brucellosis in Nigeria and its economic impacts on production. *Afr. J. Clin. Exp. Microbiol.*, 2022, 23(3):227-237.
7. Sarma O, Singh P. Brucellosis: A major abortion causing disease of livestock with zoonotic potential. *Pharm. Innov. J.*, 2022, 11(8):270-277.
8. Pereira CR, Cotrim de Almeida JVF, Cardoso de Oliveira IR, Faria de Oliveira L, Pereira LJ, Zangeronimo MG, Dorneles EMS. Occupational exposure to *Brucella spp.*: A systematic review and meta-analysis. *PLoS Negl. trop. Dis.*, 2020, 14(5):e0008164.
9. Du Preez J. How to prevent and control the spread of brucellosis: part 4 of 7. *Farmers Wkly., 2018*, 2018(18015):32-34.
10. Santos RL, Souza TD, Mol JP, Eckstein C, Paíxão TA. Canine brucellosis: an update. *Front. Vet. Sci.*, 2021, 8:594291.
11. Laine CG, Scott HM, Arenas-Gamboa AM. Human brucellosis: Widespread information deficiency hinders an understanding of global disease frequency. *PLoS Negl. Trop. Dis.*, 2022, 16(5):e0010404.
12. Avila-Granados LM, Garcia-Gonzalez DG, Zambrano-Varon JL, Arenas-Gamboa AM. Brucellosis in Colombia: Current status and challenges in the control of an endemic disease. *Front. Vet. Sci.*, 2019, 6:321.
13. Arshad R, Pal K, Sabir F, Rahdar A, Bilal M, Shahnaz G, Kyzas GZ. A review of the nanomaterials use for the diagnosis and therapy of salmonella typhi. *J. Mol. Struct.,* 2021, 1230*:*129928.

14. Addis M. Public health and economic importance of brucellosis: A review. *Pub. Health*, 2015, 5(7):68-84.
15. Al Dahouk S, Sprague LD, Neubauer H. New developments in the diagnostic procedures for zoonotic brucellosis in humans. *Rev. Sci. Tech.*, 2013, 32(1):177-88.
16. Refai M. Application of biotechnology in the diagnosis and control of brucellosis in the Near East Region. *World J. Microbiol. Biotechnol.*, 2003, 19:443-449.
17. Padilla Poester F, Nielsen K, Ernesto Samartino L, Ling Yu W. Diagnosis of brucellosis. *Open Vet. Sci. J.*, 2010, 4(1):46-60.
18. Zhang Y, Hu X, Wang Q, Zhang Y. Recent advances in microchip-based methods for the detection of pathogenic bacteria. *Chin. Chem. Lett.*, 2022, 33(6):2817-2831.
19. Kurmanov B, Zincke D, Su W, Hadfield TL, Aikimbayev A, Karibayev T, Blackburn JK. Assays for identification and differentiation of *Brucella* species: A review. *Microorg.*, 2022, 10(8):1584.
20. Markoulatos P, Siafakas N, Moncany M. Multiplex polymerase chain reaction: a practical approach. *J. Clin. Lab Anal.*, 2002, 16(1):47-51.
21. Elbehiry A, Aldubaib M, Marzouk E, Abalkhail A, Almuzaini AM, Rawway M, Draz A. The Development of Diagnostic and Vaccine Strategies for Early Detection and Control of Human Brucellosis, Particularly in Endemic Areas. *Vaccines*, 2023, 11(3):654.
22. Gul ST, Khan A. Epidemiology and epizootology of brucellosis: A review. *Pak. Vet. J.,* 2007, 27(3):145.
23. Moreno E, Cloeckaert A, Moriyón I. *Brucella* evolution and taxonomy. *Vet. Microbial.*, 2002, 90(1-4):209-227.
24. Scholz HC, Revilla-Fernández S, Dahouk SA, Hammerl JA, Zygmunt MS, Cloeckaert A, Hofer E. *Brucella vulpis sp*. nov., isolated from mandibular lymph nodes of red foxes (*Vulpes vulpes*). *Int. J. Syst. Evol. Microbiol*., 2016, 66:2090-2098.
25. Zowghi E, Ebadi A, Yarahmadi M. Isolation and identification of *Brucella* organisms in Iran. *Arch. Clin. Infect. Dis.*, 2008, 3(4):185-188.
26. Deng Y, Liu X, Duan K, Peng Q. Research progress on brucellosis. *Curr. Med. Chem.*, 2019, 26(30):5598-5608.
27. Díaz A. Epidemiology of brucellosis in domestic animals caused by *Brucella melitensis*, *Brucella suis* and *Brucella abortus*. *Rev. sci. tech. - Off. Int. Epizoot.*, 2013, 32(1):43-51.
28. Vizcaino N, Cloeckaert A, Zygmunt MS, Dubray G. Cloning, nucleotide sequence, and expression of the *Brucella melitensis omp31* gene coding for an immunogenic major outer membrane protein. *Infect. Immun*., 1996, 64(9):3744-3751.
29. Khan AU, Sayour AE, Melzer F, El-Soally SAGE, Elschner MC, Shell WS, El-Adawy H. Seroprevalence and molecular identification of *Brucella spp*. in camels in Egypt. *Microorg*., 2020, 8(7):1035.
30. Yagupsky P, Morata P, Colmenero JD. Laboratory diagnosis of human brucellosis. *Clin. Microbiol. Rev.* 2019, 33(1):e00073-19.

31. Navarro E, Serrano-Heras G, Castaño MJ, Solera JJCCA. Real-time PCR detection chemistry. *Clin. Chim. Acta*., 2015, 439: 231-250.

32. abdulkaliq Awadh H, Hammed ZN, Hamzah SS, Saleh TH, AL-Rubaii BAL. Molecular identification of intracellular survival related *Brucella melitensis* virulence factors. *Biomed.*, 2022, 42(4):761-765.
33. Hosseinidoust SR, Ahmadi A, Ahmadi Z, Hajia M, Safari Z, Golmanesh L. Detection of *Brucella abortus* by PCR assay and comparison whit culture assay. *J. Mil. Med.*, 2005, 7(3):239-244.
34. Dadar M, Alamian S, Behrozikhah AM, Yazdani F, Kalantari A, Etemadi A, Whatmore AM. Molecular identification of *Brucella* species and biovars associated with animal and human infection in Iran. *Vet. Res. Forum.*, 2019, 10(4):315-321.
35. Shafei B, Ahmadi M, Dastmalchi Saei H. Diagnosis of *Brucella abortus* and *Brucella melitensis* in the milk of cattle and sheep in Kordestan province by polymerase chain reaction. *J. Vet. Microbiol*., 2012, 2(8):127-135.
36. Aliyev J, Alakbarova M, Garayusifova A, Omarov A, Aliyeva S, Fretin D, Godfroid J. Identification and molecular characterization of *Brucella abortus* and *Brucella melitensis* isolated from milk in cattle in Azerbaijan. *BMC Vet. Res*., 2022, 18(1):1-9.

How to cite this article: Noura M, Kamaladdini H, Haddadi F, Najimi M. Molecular Identification of *Brucella* Bacteria Using *BLS* and *Omp31* Genes. *Curr. Appl. Sci.*, 2025, 3(1):11-20. https://doi.org/10.22034/cas.2023.391375.1031