Preventing Laboratory-acquired Brucellosis in the Era of MALDI-TOF Technology and Molecular Tests

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Abstract

Brucellosis is one of the most common etiologies of laboratory-acquired infections worldwide, and handling of living brucellae should be performed in a Class II biological safety cabinet. The low infecting dose, multiple portals of entry to the body, the great variety of potentially contaminated specimens, and the unspecific clinical manifestations of human infections facilitate the unintentional transmission of brucellae to laboratory personnel. Work accidents such as spillage of culture media cause only a small minority of exposures, whereas >80% of events result from unfamiliarity with the phenotypic features of the genus, misidentification of isolates, and unsafe laboratory practices such as aerosolization of bacteria and working on an open bench without protective goggles or gloves. Although the bacteriological diagnosis of brucellae by traditional methods is simple, the Gram stain and the biochemical profile of the organism, as determined by commercial kits, can be misleading, resulting in inadvertent exposure and contagion. The use of novel identification technologies is not hazard-free. The MALDI-TOF technology requires an initial bacterial inactivation step, while the instruments' reference database may misidentify *Brucella* as belonging to other Gram-negative species. The rapid identification by the FISH method mistakes brucellar isolates for members of the closely related *Ochrobactrum* genus.

Introduction

In 1997 an outbreak of brucellosis occurred among the medical personnel of the Soroka

University Medical Center (SUMC) located in the southern region of Israel were *B. melitensis* is

endemic [1]. The event originated in the clinical microbiology laboratory (CML) and involved three

laboratory technicians, two members of the administration personnel, one Infectious Diseases fellow,

and a physical therapist that made a short visit to the facility. All 7 cases were diagnosed within 6

weeks by positive blood cultures and serological tests [1]. The CML was located in the hospital's

basement and ventilation was provided by a ducted system, but the blood culture room where most

of the *Brucella* cultures were processed was not under negative pressure. Two of the affected

technicians worked in other lab rooms, as well as the administration staff.

No noticeable working accidents such as spillage of culture media or malfunction of the biological

type II safety cabinets could be recalled, but one of the affected technicians and the Infectious

Diseases fellow were seen on various occasions working on the bench without wearing protective

gloves [1]. Phenotyping of the 7 *B. melitensis* isolates -no molecular typing was available at that time-

revealed four different biotypes, indicating multiple exposures and, thus, an ongoing safety problem

[1]. Review of the laboratory records for 1997 revealed that *B. melitensis* was detected in 127 of

3,974 (3.2%) positive aerobic Bactec (Becton, Dickinson Diagnostic Instrument Systems, Towson, MD,

USA) blood culture vials and 11 of 126 (8.7%) Isolator Microbial Tube (also known as lysis-

centrifugation) cultures (Wampole Laboratories, Cranbury, NJ, USA) [1].

The described cluster of cases exemplifies many of the typical characteristics of outbreaks of

laboratory-acquired brucellosis (LAB), including non-compliance with biosafety rules in the handling

of cultures, airborne dispersion of brucellae by poorly-designed ventilation systems, and the remarkable infectivity of *Brucella melitensis,* causing multiple cases of the disease in the context of a highly endemic epidemiological background [2, 3].

In the last two decades elapsed since the SUMC outbreak, molecular detection tests that do not require the isolation of dangerous *Brucella* organisms [4-6], and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) identification technology that substantially reduces the manipulation of living bacteria [6-8] have been introduced into clinical practice. Although less hazardous than traditional bacteriological methods, the novel approaches are not entirely risk-free and present new biosafety challenges. The present review summarizes the factors involved in the causation of laboratory-acquired brucellar infections, the prevention of occupational exposures to the organism and their management, and discusses the biosafety implications of the use of the new detection and identification methods.

The organism

Brucellae are small Gram-negative facultative intracellular coccobacilli that infect a variety of feral and domestic animals [9, 10]. The genus currently comprises 12 recognized species of which four -namely *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*- are the main etiologic agents of human disease [10]. Although each one of these species is associated with preferential animal hosts (*B. melitensis* with small ruminants, *B. abortus* with cattle, *B. suis* with swine, and *B. canis* with canids), brucellae can be transmitted to other animal species, including humans [9, 10]. In most cases, the disease in men results from intimate contact with diseased animals or consumption of contaminated dairy products, and person-to-person contagion is exceptional [11].

In the past, this zoonosis had a worldwide distribution, but the implementation of rigorous control policies in industrialized countries consisting of periodic screening of livestock, culling of infected animals, and routine vaccination of herds have effectively controlled the disease. In industrialized countries brucellosis is uncommon and most cases can be traced to occupational exposure of veterinarians, laboratory personnel, and abattoirs workers, foreign travels, or illegal import of contaminated foodstuff [10]. The disease, however, remains endemic in Mediterranean countries, the Middle East, Latin America, the Indian subcontinent, and Africa where half a million new cases of human infections are annually detected [10].

Brucella species are characterized by several biological features that facilitate their easy transmission to laboratory technicians (Table 1), making the bacterium one of the most common etiologies of laboratory-acquired infections: the infecting dose of aerosolized bacteria is low, ranging from 10 and 100 organisms; the organism may penetrate the human body through portals of entry that are relevant to the laboratory work and, especially, the respiratory tract and conjunctival epithelium, but also the abraded and uncovered skin and the gastrointestinal tract; the attack rate in the CML setting is high, ranging between 30% and 100%, depending on the inoculum, the physical location of the workers, and the source of the exposure [13, 14]; it grows on routine culture media such as blood- and chocolate-agar and colonies exhibit an indistinctive appearance; although the organism does not produce spores, it may persist on inanimate surfaces for weeks and even months [14]; decontamination of contaminated equipment with 70% ethanol or bleach is only mildly effective [14, 15].

Human Brucellosis, a "Great Imitator"

Human brucellosis exhibits a wide range of clinical severity, spanning from asymptomatic infections and "flue-like disease" to life-threatening meningoencephalitis and endocarditis [10]. The disease may manifest as a systemic febrile illness or a focal infection affecting different body organs and systems such as the joints and bones, the liver, the genital tract, *etc.* [9, 10]. *Brucella* infections may mimic a variety of infectious and non-infectious conditions, and the true nature of the disease may not be suspected and the diagnosis delayed or missed altogether. Even in areas endemic for the disease the diagnosis of brucellosis is not initially considered in a substantial fraction of patients [16]. Under these circumstances, the physicians may fail to alert the clinical laboratory that the patients' specimens might contain a hazardous pathogen and should be handled with appropriate safety precautions [2]. The vague and unspecific manifestations of the disease may also result in a delay in the recognition of outbreaks of LAB [3] and failure to implement corrective measures and prevent additional cases. Another implication of the protean manifestations of human brucellosis is the wide variety of clinical specimens that can harbor *Brucella* organisms and be submitted to the lab. Although blood and synovial fluid aspirates are the most frequently contaminated samples, biopsy material, bone marrow, cerebrospinal fluid, urogenital specimens, placentae, and amniotic fluid may also represent unforeseen sources of occupational exposure [17].

Whereas in the United States, approximately 120 cases of brucellosis are reported annually countrywide and LAB events are rare [3], in a single clinical laboratory in Ankara, Turkey, a mean of 400 clinical specimens yield *Brucella* organisms each year, and LAB affected 10 of 55 (18%) technicians, with an annual risk of 8% per employee [18].

The incubation of the disease in humans is highly variable, spanning from a few days to months,

and 21% of LAB cases have onset >12 weeks after exposure, implying that exposed personnel should

be closely followed-up for the appearance of symptoms of the infection and seroconversion for a

prolonged period [3].

Diagnosing Human Brucellosis

A prompt and clear-cut diagnosis of human brucellosis is critical for the patient's management

because successful antibiotic therapy requires prolonged administration of drug combinations that

are not employed for other infections, and unless the organism is eradicated at the early stages of

the disease, brucellosis may run a chronic and complicated clinical course [10]. Besides, the diagnosis

of brucellosis in man has serious public health significance because it implies contact with a zoonotic

source that has to be traced, identified, and controlled, or could represent a bioweapon attack [19,

20].

The laboratory confirmation of the diagnosis has traditionally relied on the cultural isolation and

identification of the agent by biochemical means, and/or positive serological tests.

The isolation of brucellae remains a suboptimal diagnostic tool. The bacterium is slow-growing

and the sensitivity of the culture is substantially reduced in protracted and/or focal infections [21]. In

recent years, novel culture-independent nucleic acid amplification tests (NAATs) have been added to

the diagnostic armamentarium, enabling rapid detection, speciation, and biotyping of the organism

[6]. The recovery of the organism, however, has not been abandoned, and the isolation of brucellae

from blood, other normally sterile body fluids, and tissues are the only irrefutable proof of active

infection [9]. From the epidemiological point of view, isolation enables speciation and genotyping,

making it possible to track the source, and discriminates between wild and vaccine strains [22]. A

positive culture is also important for the diagnosis at the initial stages of the infection, when the

results of the serological tests are still negative or show non-diagnostic antibody titers [23], and

enables the performance of antibiotic susceptibility testing of the isolate when indicated. An

important benefit of isolation is the fact that it establishes the diagnosis in cases in which the disease

is not clinically suspected. Not uncommonly, the bacterium is unexpectedly recovered from a blood

culture obtained as part of the routine workup of a febrile patient [16, 24], whereas ordering a

serological assay or a species-specific NAAT requires considering *a priori* the possibility of brucellosis.

The diagnosis of the disease by serological means has the advantages of simplicity and low cost,

which are especially relevant to endemic and remote rural regions where more sophisticated and

expensive tools are scarce or non-existent [6]. The approach, however, has several drawbacks: it has

low sensitivity in the initial stages of the infection, protracted cases, and focal infections; the

specificity is limited by cross-reacting antigens of taxonomically-related and unrelated bacterial

species; interpretation of the serological test results may be difficult in individuals repeatedly

exposed to the organism [6].

Although the NAATs have an unsurpassed sensitivity, the high cost and unavailability of

sophisticated molecular technology in resource-poor endemic areas, as well as the lack of

standardization and reproducibility of the different methods and commercial kits limit their routine

use [6]. Also, a positive NAAT cannot discriminate between active disease and past and resolved

brucellar infection [6].

Brucella Cultures and Laboratory Safety

The concentration of viable brucellae in blood and other clinical samples is variable, ranging from 1 colony-forming unit (CFU) /mL to >1,000 CFUs/mL, being higher in the early stages of the disease, and decreases over time as the result of a mounting immune response [25, 26]. Whereas grinding and homogenization of tissues are risky procedures that must be performed in a safety cabinet, normally sterile body fluids other than reproductive specimens (amniotic fluid, placental products) are not considered to represent a tangible risk of transmission to laboratory personnel unless a flagrant breach of laboratory safety practices has been committed. The risk of contagion, however, increases exponentially during and after incubation of the cultures on solid or liquid media, and colonies growing on an agar plate and positive blood culture vials contain millions of living and highly transmissible bacteria.

Overall, 142 of 167 (85%) laboratory workers exposed and 46 of 71 (65%) LAB cases reviewed by Traxler *et al*. occurred in CMLs, followed by research and reference labs, and vaccine production facilities [3]. Laboratory accidents such as breaking of centrifuge vials [27] or blood-culture bottles [28], self-inoculation of a suspension of brucellae [29] or a patient's synovial fluid [28], or spillage of culture broths play a minor role in LAB events and caused only 18 of 165 (11%) exposures [3]. More commonly, the transmission is the result of unsafe working practices, such as handling of culture media on an open bench [12, 28, 30-32], not using protective equipment [18]; sniffing plates [13, 18, 33-35], or ingesting suspensions of living brucellae during mouth pipetting [29].

Disregarding the portal of entry to the human body, brucellae are translocated to the regional lymph nodes and subsequently transferred to the bloodstream causing continuous bacteremia and invasion of macrophages-rich body tissues and organs, such as the bone marrow, lymph nodes,

spleen, and liver where they live an intracellular lifestyle [9]. Therefore, blood cultures are suitable

specimens for detecting circulating brucellae, especially at the initial stages of the infection. Blood

samples are also easy to obtain and repeat, and drawing multiple specimens increases the detection

sensitivity [6]. Blood samples are, thus, the most common clinical specimens from which brucellae

are isolated in the CML and, thus, represent the most common source of LAB.

Modern automated blood culture systems detect the presence of microorganisms by continuous

monitoring of rising CO_2 levels in the inoculated vials released by multiplying bacteria or fungi [6].

The measurement is performed without piercing the vial top and, thus, no nebulization of viable

bacteria occurs. However, once the CO_2 level reaches the positivity threshold, the broth is aspirated,

subcultured on solid media and incubated, and a Gram stain is performed [6]. Bacterial colonies

developing on the agar surface are then subjected to a variety of biochemical tests to identify the

isolate. Bacteriological procedures such as centrifugation and vortexing of bacterial suspensions, the

performance of subcultures, and biochemical testing may also result in dispersion and spillage of

living bacteria, contamination of the laboratory environment, and unintentional transmission to the

working personnel. Although it has been traditionally that *Brucella* species are slow-growing bacteria

[3], modern automated blood culture systems enable the detection of members of the genus within

the routine one-week incubation period [6, 37]. Therefore, a short time-to-detection does not

reliably exclude the presence of *Brucella* organisms in the blood culture vial.

Because *Brucella* organisms undergo phagocytosis and tend to circulate in the bloodstream

inside polymorphonuclear (PMN) cells, the use of the Isolator Microbial Tube was traditionally

considered preferable to other blood culture methods for the detection of brucellae in blood

samples [9, 10]. Blood specimens are seeded into special vials that contain a mixture of

anticoagulant to prevent clotting and a detergent that disrupts the cellular membranes of PMNs,

releasing phagocyted but still viable microorganisms. The resulting lysate is then centrifuged and the

sediment is dispersed onto appropriate agar plates and incubated. Naturally, the manipulation of the

specimen and the examination of the Petri dishes for the presence of growing colonies, even if

performed in a biological safety cabinet, imply a substantial transmission hazard for the laboratory

personnel.

To avoid exposure, the Centers for Disease Control and Prevention (CDC) have strongly

recommended that all laboratory procedures with living brucellae require level 3 biosafety

precautions. Handling of the organism should be performed in Class II biological safety cabinets by

technicians protected by gloves, goggles, and a respiratory mask [36]. The drawback of this approach

is that, by the time bacterial isolates are suspected or confirmed as *Brucella* species, careless

extensive work with the organism has usually taken place, and inadvertent exposure of technicians

may have already occurred. Following the 1997 SUMC outbreak of LAB, strict infection control

practices have been rigorously implemented [1]. All blood culture vials flagged positively by the

automated Bactec instrument are manipulated in biological safety cabinets until the possibility of a

Brucella species is firmly ruled-out. Plates are sealed when not in use and properly disposed of and

sterilized as soon as the work with them has been completed [30]. Since the antibiotic resistance

pattern of the genus is predictable and acquired resistance is uncommon, susceptibility testing of

identified *Brucella* organisms has been stopped altogether. Because a prospective comparison of the

performance of the Isolator Microbial Tube and the safer automated Bactec system for detecting

*Brucell*a bacteremia demonstrated a statistically significant advantage of the latter in terms of both

sensitivity and time-to-detection [37], the use of the lysis-centrifugation system for patients with suspected brucellosis has been utterly discouraged.

Since the implementation of this enhanced safety policy, no further cases LAB have been detected in more than 20 years, despite an ever-growing number of isolations [38]. It seems, then, prudent, to recommend that in areas endemic for the infection, all positive blood culture vials should be initially processed in safety cabinets, pending final identification of the isolate. Since CML technicians in these regions frequently handle *Brucella* organisms, the performance of serological tests may facilitate the distinction between old and newly-acquired infections. A baseline serological test should be performed upon personnel recruitment, followed by periodic serological monitoring.

Phenotypic Identification of Brucellae

The presumptive identification of members of the genus *Brucella* relies on the typical Gram staining appearance, positive oxidase, catalase, and urease activity, no fermentation of sugars, and lack of motility, and should be confirmed by a molecular method or by a positive slide agglutination reaction with antiserum against the bacterial O-lipopolysaccharide [6]. Each one of the individual links of this chain is prone to error, misidentifying the isolate and causing LAB. Besides, because of the effective veterinarian control of the zoonosis, the disease has become uncommon in industrialized countries, and personnel working at CMLs have become unfamiliar with the phenotypic characteristics of the genus [2].

The Gram stain plays an early and key role in the correct identification of *Brucella* species. The presence of small Gram-negative coccobacilli should be the first hint of the true nature of the

unknown organism, and no biochemical, MALDI-TOF or molecular testing should be ever carried-out before a thoughtful Gram staining examination of the isolate has been performed. A poor staining technique may result in the classification of brucellae as Gram-positive organisms that can be mistaken for streptococci [2].

The identification of *Brucella* species by conventional manual methods takes a few days in the course of which it may potentially expose the laboratory personnel to a highly transmissible organism. Routine bacteriological procedures such as the centrifugation and vortexing of bacterial suspensions, the performance of subcultures. and biochemical testing may cause aerosolization and spillage of bacteria, and the contamination of the laboratory environment and bench surfaces. Specially hazardous is the catalase test, which is strongly positive in all *Brucella* species and produces bubbling and nebulization of living bacteria [12].

These traditional identification methods have been gradually simplified by commercial systems that save considerable labor time. These kits consist of panels of ready-made dried chemical substrates that, once inoculated with suspensions of the unknown bacterium and incubated, identify the isolate by comparing the test results with those of a comprehensive database. Because of the similarity of the biochemical profiles, these systems do not discriminate between true brucellae and other members of the *Brucellaceae* family and, particularly, the *Ochrobactrum* species *(O. anthropi* (39, 40] or *Ochrobactrum intermedium* (39-41), as well as the taxonomically unrelated *Haemophilus influenzae* (42), *Bergeyella zoohelcum* (43), *Bordetella bronchiseptica* [44], or *Psychrobacter phenylpyruvicus* (formerly *Moraxella phenylpyruvica)* [45]. These unfortunate mistakes have already caused outbreaks of LAB [46] and, therefore, any of these uncommon bacterial species identified by

phenotypic methods should be considered a potential *Brucella* organism and, as such, carefully handled in a safety cabinet until this possibility is excluded. The familiarity of CML personnel with the microbiological features of brucellae, the safe handling of culture media, and the pitfalls in the identification of members of the genus should be improved, refreshed, and maintained through periodic education.

The recent introduction of MALDI-TOF-based instruments in the clinical microbiology laboratory has deeply changed the way microorganisms are identified. MALDI-TOF technology enables the fast (within minutes), precise, reproducible, and cost-effective identification of bacterial isolates to the species level, substituting the manual, and slow traditional biochemical testing [7, 47].

The use of novel technology for the identification of brucellae, however, is not risk-free and the procedures recommended by the manufacturers for other bacterial pathogens are not adequate for the manipulation of biosafety level 3 *Brucella* strains [15]. In a large survey of exposure to the organism among laboratory personnel of New York City hospitals, inappropriate use of the MALDI-TOF and misidentification of the isolate were responsible for 84% of the events [2], and represent potential health hazards for laboratory workers. MALDI-TOF MS analyses should never be performed directly to bacterial colonies growing on agar plates or positive blood culture broth before a Gram stain of the isolate is examined and other phenotypic features, such as colony morphology, growth conditions, and media, are taken into consideration [48]. If small Gram-negative coccobacilli are visualized, and strict aerobic growth on blood-agar and chocolate-agar media, capnophilia, and white, non-hemolytic colonies are detected, a *Brucella* species should be suspected. In CMLs where positive blood culture vials broth is directly transferred to the MALDI-TOF matrix to save time, occupational

risks from unidentified clinical isolates may occur [2].

To avoid exposure to living brucellae, initial bacterial inactivation step is mandatory before the protein extraction. This can be accomplished with either 33% acetonitrile, 33% absolute ethanol, 3% trifluoroacetic acid, and 31% water [15]; absolute ethanol and formic acid (v/v 10%) [8]; or absolute ethanol, 70% formic acid, and acetonitrile [49, 50].

The capability of MALDI-TOF technology to correctly identify brucellae is evolving at a slow pace. Since commercial MALDI-TOF instruments are costly and unavailable in Developing World countries where the zoonosis is endemic, data based on the field evaluation of the method are scarce. Initially, the database reference of the Vitek MS system (bioMérieux, France) misidentified *B. melitensis* as *O. anthropi* [51], and that of its competitor, the Bruker system (Bruker Daltonics, Germany), unreliably differentiated between *Brucella* species [8, 48]. The use of an improved Vitek MS reference database made it possible the unambiguous discrimination between members of the genus *Brucella* and *Ochrobactrum* species, as well as satisfactory speciation of the three most common zoonotic species: *B. melitensis, B. abortus,* and *B. suis* [52]. It is to be expected that increasing experience MALDI-TOF-based instruments will further improve the identification and speciation of brucellae and replace the slow, labor-intensive, and unsafe manual procedures.

Identification by Molecular Methods

Fluorescence *in situ* hybridization (FISH) assay targeting a segment of the 16S rRNA gene has recently been introduced into clinical practice. The novel test can be applied directly to positive blood culture broths enabling the rapid identification of all *Brucella* species pathogenic to humans [53] However, because of the low polymorphism of the "universal" 16S rRNA gene sequence among

members of the *Brucellaceae* family, the test cannot differentiate brucellae from *Ochrobactrum* species [54]. Therefore, bacterial isolates presumptively identified as *Ochrobactrum* species by either MALDI-TOF technology or the FISH assay should be initially processed in class II safety cabinets until the possibility of brucellae has been ruled out.

Post-exposure Prophylaxis and other Measures

Following the recognition of the exposure incident, a thorough investigation should be immediately conducted. The event should be reconstructed, documented and reported to the Public Health authorities; the timing, setting, and circumstances of the exposure event should be determined as precisely as possible, and the results of the investigation should be discussed with the laboratory staff and used for educational purposes and correction of deficiencies; the members of the laboratory personnel potentially involved in the exposure should be identified, and the individual risk should be assessed as high or low following the Centers for Diseases Control and Prevention guidelines [55], as condensed in Table 2. Because of the high infectivity of *Brucella* organisms, the attack rate of clinical disease among exposed laboratory personnel is remarkably high, and 71 LAB cases were diagnosed among 167 exposed workers reviewed by Traxler *et al.* [3]. Therefore, post-exposure prophylaxis consisting of doxycycline (100 mg) orally twice daily and rifampin (600 mg) once daily for a minimum of 21 days should be offered to those considered to be at high-risk for LAB, as well as for pregnant women and immunosuppressed individuals. Trimethoprim-sulfamethoxazole (cotrimoxazole) or another antimicrobial agent effective against *Brucella* should be selected (for at least 21 days) if doxycycline or rifampin are contraindicated. Serological testing of all the exposed laboratory personnel should be performed as soon as possible and repeated at 6 weeks, 12 weeks,

18 weeks, and 24 weeks after the last known exposure [2, 55], as well as monitoring of clinical

symptoms and signs, disregarding their risk-assessment classification.

Conclusions

Brucellosis continues to be a public health problem of worldwide dimensions that poses a

tangible risk of transmission to laboratory workers. In regions endemic for the zoonosis, the hazard

of contagion of the clinical microbiology laboratory personnel may be high, but even in countries

where strict control measures are implemented and the zoonosis has been controlled, the accidental

transmission of *Brucella* organisms remains a serious concern. Exposure to virulent brucellae may

occur at each one of the successive steps of the diagnostic chain, from handling of clinical specimens,

through isolation of the organism, and to its final identification. Although improved culture

techniques and novel detection and speciation methods have been added to the clinical

microbiology laboratory armamentarium, *Brucella* species still pose a tangible threat to working

personnel. To avoid occupational infections in today busy and complex laboratory environment, a

comprehensive approach is necessary, consisting of education of technicians on the microbiological

identification of *Brucella* species and its pitfalls, and strict adherence to safe work practices and

proper use of containment devices and personal protective barriers during the manipulation of

bacterial isolates.

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Serological testing of all the exposed personnel should be performed as soon as possible and repeated at 6 weeks, 12 weeks, 18 weeks, and 24 weeks after the last known exposure [2, 55, 56], as well as monitoring of clinical symptoms and signs, disregarding their risk-assessment classification.

Category	Hazard	
Bacteriological features of brucellae	Low infecting dose	
	Multiple portals of entry to the human body	
	High infectivity	
	Long-term persistence on inanimate surfaces	
	Relative resistance to decontamination	
	Growing biomass after incubation	
Epidemiology	High burden of disease in endemic areas	
Clinical disease	Unspecific symptoms and signs	
	Mimics other infectious and non-infectious conditions	
	Lack of communication with the laboratory	
	Contamination of a wide diversity of clinical specimens	
Identification of the isolate	Unfamiliarity with the genus in non-endemic regions	
	Inconspicuous appearance of colonies	
	Misleading Gram stain	
	Misidentification by	commercial biochemical kits
		MALDI-TOF technology
		molecular methods
Unsafe laboratory practices	Lack of biosafety protocols	
	Lack of personal protective equipment	
	Work in an open bench	
	Eating, drinking, or smoking at the work station	
	Aerosolization by centrifugation, vortexing, catalase test, *etc.*	
	Accidents (spillage of media, breakage of tubes, needle stick injuries, *etc.*)	
Environment and equipment	Crowding	
	Poorly-designed ventilation systems	
	Malfunction or improper use of safety cabinets	

Table 1. Hazards involved in laboratory-acquired brucellosis.

Risk Category	Exposure setting					Post exposure measures		
	Enriched material[a] and reproductive clinical specimens				Other clinical specimens			
	Work outside of a CCBSC[b]	Work at <5 feet from someone working outside a CCBSC[b]	Work on a CCBSC[b] without PPE[c]	Aerosol-generating procedures on an open bench	Contact with mucosae or broken skin	Post-exposure prophylaxis	Serological follow-up	Clinical monitoring
High	Yes	Yes	Yes	Yes	Yes	Yes	6 months	6 months
Low	No	No	No	No	No	No	6 months	6 months

[a]: Contact with *Brucella* isolates and cultures on solid or liquid media
[b]: Certified Class II biological safety cabinet
[c]: Personal protective equipment (gloves, gown, eyes protection)

Table 2. Assessment of the exposure risk and indications for post-exposure prophylaxis and monitoring.