Mashhad University of Medical Sciences (MUMS)

Reviews in Clinical Medicine

Clinical Research Development Center Ghaem Hospital

Current Status and Future Prospect of Brucella Blood Culture in Iran: A Review of the Recent Findings

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ARTICLE INFO

Article type
Review article

Article history
Received: 12 Feb 2019
Revised: 25 Feb 2019
Accepted: 28 Feb 2019

Keywords
Brucella
Blood Specimens
Diagnosis

ABSTRACT

The prevalence of brucellosis has increased in recent years in some regions in Iran, particularly in the western, northeastern, and some central areas. Undoubtedly, the main causes of brucellosis are the lack of vaccination coverage in livestock and distribution of dairy products. In addition, attention must be paid to the diagnostic difficulties associated with slow growth specificity and use of inefficient methods, which lead to the delayed diagnosis of the disease. All the available diagnostic procedures are currently used for the diagnosis of brucellosis, including isolation on culture media, serological procedures, and molecular techniques. Among these methods, isolation on culture media has shown the minimum efficiency, especially in blood specimens, which are the most commonly requested specimens in disease diagnosis. The influential factors could be the use of unapproved commercial kits, applying outdated diagnostic procedures, and using unqualified specimens in hospitalized patients. The present study aimed to enhance the current status of the isolation method, especially in the endemic areas for brucellosis. Several parameters were assessed in this regard, including the role of laboratory conditions, sampling quality, type of culture media, and various isolation methods, in order to review the studies aiming to increase the efficiency of this method.

Please cite this paper as:
Hajia M. Current Status and Future Prospect of Brucella Blood Culture in Iran: A Review of the Recent Findings. Rev Clin Med. 2019;6(1):24-28.

Introduction

Brucellosis is considered to be a major health concern across the world, especially in developing countries (1-5). The diagnosis is difficult due to the unspecific signs and symptoms of brucellosis. Reports have confirmed that the majority of the patients with brucellosis are not diagnosed within the early stages of the disease due to the use of inefficient laboratory methods. This issue has also been confirmed considering the numerous chronic forms of the disease by hospitalized patients (6-8).

Serological methods are effective in the diagnosis of brucellosis if the defective parameters are eliminated, especially in rural areas. However, the types of new strains and dominant serotypes must be identified for epidemiological purposes and determining the patterns of antibacterial resistance. Moreover, an accurate diagnosis is only possible through the isolation of Brucella species.

Recent reports have denoted the challenges associated with the diagnosis of brucellosis (9-11). Most blood specimens are tested at health laboratory centers (HLCs) and hospital laboratories in every country, with the exception of private clinical laboratories, which often receive few specimens (12). The diagnosis of brucellosis is based on classic standard tube agglutination protocols in HLCs, while hospital laboratories are also required to culture the blood specimens obtained from admitted patients. Molecular techniques are

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mainly applied in private laboratories. Despite the introduction of new techniques, these centers use traditional methods, which have low sensitivity and specificity in Iran (13-15). Undoubtedly, the protocols that are currently applied need proper revision in HLCs and hospital laboratories. The isolation of organisms is considered to be the 'gold standard' for the diagnosis of Brucella species.

Each year, several studies are conducted in this regard in Iran; however, none has properly identified the involved strains in the endemic areas for brucellosis. These studies have been based on only a few selected specimens, not being able to accurately determine the dominant serotypes or show the pattern of antibacterial resistance in Brucella species since there have been no recovered organisms to be the representative of the disease outbreak.

In Iran, no reports have been published regarding the use of blood specimens with proper sensitivity. The isolation procedure of the slow-growing and fastidious Brucella species is extremely difficult and time-consuming, especially in blood specimens. The need to improve isolation conditions has urged researches to perform numerous studies in this regard. As such, various influential factors have been identified, such as enhancing the quality of culture media, introducing new procedures with higher sensitivity, and safe working conditions (16,17).

The present study aimed to review the use of blood specimens in culture procedures, attempting to indicate the opportunities ahead to increase the success rate of this process.

Literature Review

Biosafety Requirements

Although culture is considered to be the diagnostic method of choice for brucellosis, it involves the risk of infection in laboratory personnel and requires special precautions in the laboratory. Numerous reports have been released regarding post-exposure recommendations and laboratory safety measures (18-20). However, laboratory-acquired brucellosis has been frequently reported in the previous studies in this regard (21-23). The laboratory staff who are engaged in the identification of Brucella should be aware that the infectious dose is 10-100 bacteria, and the personnel are at a high risk of accidental infection. Consequently, brucellosis remains one of the most commonly reported laboratory-acquired infections despite the reports in this regard (24).

Auditing reports have revealed that many laboratories do not adopt proper safety precautions in the diagnosis of brucellosis (25). Moreover, facility assessments have indicated that in many laboratories the, the biosafety status of brucellosis diagnosis does not correspond to the requirements, even to the minimum standard for Brucella culture.

In the United States, approximately 120 cases of brucellosis occur each year (26). However, no national surveillance systems are available for the identification of the laboratory-acquired cases. Therefore, the annual incidence of brucellosis due to laboratory transmission seems to have been under-reported. In laboratory-acquired infections, B. melitensis has been reported to be the main causative agent in 80% of the exposures, which is also associated with the significantly higher risk of exposure in laboratory staff compared to other individuals (27). Most of the references in this regard have urged the adoption of level-three biosafety requirements in the laboratories performing tests on B. melitensis and B. suis, especially in packaging and shipping tasks.

Conditions Requiring Isolation

Brucella species could often be isolated from the blood, bone marrow, wounds, pus, tissues, joints, and cerebrospinal, pleural, and ascetic fluids (28,29). The amount of the organisms and recovery rate of the culture from blood specimens are extremely low. In this regard, a conventional method based on the use of biphasic Ruiz-Castaneda bottles supplemented with 5% CO2 is applied to provide safe working conditions. However, this method has a long incubation time and provides variable rates.

Selective media are not required for the culture of the specimens obtained from the blood and other body fluids if the specimens are collected with aseptic precautions. Researchers have often recommended that growth factors or supplements be added to the culture media. Furthermore, the use of selective media has been suggested to enhance the growth rate, while none of these methods has been reported to clearly enhance the isolation rate (26). Blood cultures of Brucella species are expected to become positive within 7-21 days of incubation and should be preserved for a minimum of 45 days prior to be considered negative.

Influential Factors in the Sensitivity Rate

Brucella species have long incubation periods with poor sensitivity due to their extremely slow growth. In addition, they require enriched media with strict conditions (30,31). Low sensitivity depends on numerous factors. Brucellosis has various clinical manifestations. Therefore, the phase in which the specimens are collected is considered critical as a limited time of bacteremia could yield false negative results (32,33). Among the other in-

fluential factors in sensitivity are the low concentration of bacteria in blood specimens, previous administration of antibiotics, quantity of pathogens in clinical samples, applied culturing methods, type of strains (e.g., B. melitensis is isolated more easily from clinical specimens compared to the B. abortus cultured from clinical samples), which affect the success rate of isolation (34-38). Another important influential factor in this regard is the qualification of laboratory personnel since Brucella species are fastidious organisms and easily aerosolized, which is associated with higher working hazards (39,40).

Considering the mentioned limitations, we should not expect to have high sensitivity even under optimal conditions since the reported sensitivity of the biphasic Ruiz-Castaneda system is less than 20% although it could be higher in an acute form (41). In Iran, there is limited research on the isolation of organisms, with the estimated sensitivity reported to be less than 4% (42,43).

Previous studies in this regard have also investigated the effect of proper sampling on sensitivity, denoting that this rate could increase to 16% using current commercial biphasic medium in hospitalized patients (44).

Other Culturing Procedures

Lysis centrifugation is another procedure that is based on the analysis of erythrocytes in a citrate solution (44-46). For this purpose, a blood specimen is washed with double distilled water (DDW). Afterwards, the specimens are mixed with an equal volume of DDW and 1.5 milliliters of 4% sodium citrate and centrifuged for 30 minutes at 2000x g. Following that, the supernatant is discarded from the tube, and the sediment is inoculated on brain heart infusion agar or blood culture agar with or without CO2 for seven days. Finally, the plates are observed daily to monitor growth.

The clot culture technique involves preservation in sterile, screw cap plastic tubes containing glass beads. Blood specimens are disrupted by shaking the tube on a shaker for 15 minutes after the removal of the serum. The disrupted clot inoculates on the Castaneda medium and incubated at the temperature of 37oC with 10% CO2 for a minimum of four weeks (4). The washing procedure or clot culture method has the preference of shorter isolation time over direct inoculation as recommended in a number of references. However, these methods are associated with a higher risk of contamination, and they are not considered for diagnostic purposes in the laboratory (28,30).

Recent Trends in Isolation Techniques

Automated culture systems have replaced the traditional biphasic Ruiz-Castaneda system since they are considered to be safer and faster diagnostic methods, enabling the operator to constantly monitor blood culture systems in order to obtain higher yields and accelerate the detection of bacterial growth within less than one week. Furthermore, these systems have significantly reduced the time required for blood specimens, so that Brucella species could be detected in the blood specimens of infected patients after four days or less (46-49). This period results in a significantly higher isolation rate compared to the routine approaches.

Some of the current automated culture systems include BACTEC and BacT/Alert, which continuously measure the release of CO2 in growing microorganisms (10). Another automated system is API20NE, which has been reported to cause misidentification (50). The detection rate in the mentioned systems has been estimated at 80-100%.

Using automated systems has been associated with a relatively lower detection rate in hospitalized patients compared to outpatients. In a comparative study, the efficiency of BACTEC (Difco Laboratories, Inc., Sparks, Md) was reported to be 31.11% in this group of patients (31). However, the use of these systems is costly in referral centers. It is assumed that the positive rate associated with automated systems could increase to 90% in acute cases.

Necessity of a Revised National Plan

Despite the increased incidence of Brucella species in endemic areas, their frequency has not been clarified in humans and animals in Iran (46). Currently, isolation methods are not applied in laboratories under the supervision of Jahad Keshavarsi Ministry (JKM) for livestock. Furthermore, the HLCs that deal with human infections do not use these diagnosis methods. It seems that isolation techniques still have two main limitations in the healthcare centers in Iran; the first limitation is the requirement of strict biosafety conditions, and the other limitation is the low sensitivity of these methods. Consequently, HLCs only use conventional serological tests, which are based on agglutination. Some hospitals and private clinical diagnostic laboratories may have occasionally been asked for culture blood specimens. However, reports indicate that the recovery rate of isolation in these cases is extremely low (43,44), making these methods impractical for medical decision-making.

As mentioned earlier, there are few referral laboratories that are able to use new automated procedures (25). These facilities are not cost-effective for all specimens in various regions of Iran. Therefore, these laboratories must be equipped

with modern procedures, including new automated systems.

Use of isolation procedures with new techniques has other benefits as well. For instance, they provide a clear viewpoint regarding the endemic strains in each area. Identification of new or re-immersion types and evaluating antibiotic resistance are also possible since no new research has been conducted in this regard. As a result, developing reliable molecular typing methods and molecular antibiotic resistance protocols must also be prioritized in referral centers.

The exact frequency of dominant Brucella species in various regions of Iran remains unknown, and it is not clear whether there are newly emerging types due to the transfer of livestock from the neighboring countries. Despite numerous preventative and controlling programs, no clear reports are available from the responsible governmental organizations. Adequate knowledge of the dominant species in various regions in Iran is the key to the prevention and control of newly emerging Brucella species (51-53).

Conclusion

Currently, isolation is requested for only two non-blood specimens. According to the results of the present study, lack of efficient isolation procedures confirms the necessity of revising the diagnosis network and applied procedures since the required, strict biosafety conditions are not available in most diagnostic laboratories. On the other hand, replacing conventional methods with automatic techniques and specifying the reference centers could positively affect the economic losses in animal husbandry and prevent the increased prevalence of human diseases. Moreover, such modifications help researchers to be informed of the dominant types of each endemic area and assess antibacterial resistance.

Acknowledgements

None.

Conflict of Interest

The authors declare no conflict of interest.

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