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Molecular Diagnosis of Brucellosis: A Brief Report

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Abstract

Amplification of nucleic acid by polymerase chain reaction (PCR) assays in order to diagnose infection by *Brucella* spp. has been used for more than two decades. Human Brucellosis is an endemic disease in many countries worldwide and often poses diagnostic puzzles. The implementation of PCR (standard, real-time and multiplex) can help in the accurate differential diagnosis and distinguish between acute, subacute and chronic cases. PCR has also been used for follow up of patients and serotype identification of *Brucella* spp. Overall PCR is a promising and reliable technique for the diagnosis of Human Brucellosis. Purpose of this brief report is to identify possible alternatives for rapid and accurate diagnosis of Brucellosis using PCR.

Keywords: Polymerase chain reaction; Human Brucellosis; *Brucella* spp; Molecular diagnosis

Introduction

Brucellosis is a common zoonoses of global distribution [1]. New incident cases are estimated around 500.000 each year worldwide, whereas prevalence is approximately 10/100.000 people in endemic areas. Th disease is caused by *Brucella* spp. and most usual isolated serotypes are Melitensis, Abortus and Suis. Brucellae are Gram negative intracellular bacteria that can multiply within phagocytic cells with human beings acting as end hosts. Pathogens enter the human body through ingestion, inhalation, the conjunctiva or skin abrasions. Th pathogens reside in the reticuloendothelial system, granulomas are formed and finall bacteraimia may follow. Th mechanisms leading to intracellular killing of the host immune system are unknown [2]. Brucellosis can present as acute, subacute or chronic disease with symptoms according to the various affecte organs [3]. Common symptoms include arthralgias, fever, sweating, lack of appetite, weight loss and low back pain. On clinical presentation patients ofte present with splenomegaly, hepatomegaly or both, cervical lymphadenopathy and peripheral arthritis [2,3]. Neurobrucellosis and endocarditis are the two potentially life threatening localizations of brucellosis, but osteoarticular, genitourinary and gastrointestinal involvement are far more common. Laboratory diagnosis is usually made either by isolation and culture of the microorganism or by serology tests [4]. Rarely, some patients with brucellosis will have a positive blood culture in the absence of positive serology. Th automated continuously monitored blood culture systems have shortened the time to diagnosis to a maximum of 2 weeks (mean days to results 4-5). Serology methods include the well-studied and established Rose Bengal and Wight Coombs agglutination tests; both have limitations due to low predictive values [4-6]. Newer tests such as Brucellacapt and detection of IgG and IgM antibodies by ELISA are not available worldwide due to lack of resources [6]. However, diagnostic puzzles are always present and difficulti have been noted: Cultures require level 3 biocontainment facilities and highly skilled technical personnel to handle samples and live bacteria for eventual identificatio and biotyping. Serology cannot be used for diagnosis alone in endemic areas and a verificatio test is ofte required, either cultures or repeating the tests a few days later [2-7]. Polymerase chain reaction (PCR) methods have been implemented in order to enhance sensitivity and produce quicker results [8]. Nucleic acid amplificatio techniques are now quite widely used, although no single standardized procedure has been adopted. More than 400 reports have been published describing various PCR based methods for the diagnosis of Brucella infections [7]. We provide here a brief review of the proposed methods and an appraisal of their usage in clinical settings for diagnosing Brucellosis.

Molecular Methods

Standard PCR

Th firs reports for implementation of PCR for diagnosis of Brucellosis date from the early 90's. [9-11]. Blood is the preferred tissue for the extraction of DNA, [12] however various others tissues such as serum, semen, or synovial flui have been used [13]. New approaches include the washing of blood a few times with water in order to avoid contamination with hemoglobin. Such techniques improve the sensitivity of the procedure [14]. Th standard PCR assays include one pair of primers which is used to amplify the target genomic sequence of *Brucella* spp. Pairs used include the primers for sequences encoding 16S rRNA [15,16], outer membrane protein (omp2a, omp2b and omp31) [17,18], 31 kDa immunogenic *Brucella abortus* protein (BCSP 31 B4/B5) [19-21], 16S-23S ribosomal DNA interspace region (ITS66/ ITS279) [22,23] and insertion sequence (IS711) [24,25]. Thes reports showed an excellent sensitivity for the diagnosis not only of the acute disease but also for the follow up pf patients and the detection of relapses where serology and cultures are ofte negative [26-28].

Real time PCR

Real time PCR for brucellosis has also been used. Th ability to measure DNA copy number and mRNA expression levels together with the rapid detection and differentiatio of *Brucella* spp. and the decreasing prices have made the procedure attractive and accessible

[29]. Real-time PCR seems to be highly reproducible, rapid, sensitive and specific Additionally, this assay is easily standardized and the risk of infection in laboratory workers is minimal [8]. Samples that have been tested by real time PCR include cultured Brucella cells [30], serum [31], blood and paraffin-embedd tissues [32]. Some researchers even propose the use of real-time PCR for the diagnosis of human brucellosis in everyday clinical practice since various reports give a time to fina results of only 30 minutes [30]. It is also suggested that it is the method of choice for the discrimination among inactive, seropositive and active states in testing serum samples for subjects whose clinical finding are known [32]. Th predominance of real-time PCR in terms of sensitivity and specificit is well documented in various studies. Queipo-Ortuno et al. [31] performed real-time PCR with SYBR LightCycler Green I in blood cultures of serum samples and whole blood of patients with brucellosis using primers B4 and B5 (targeting bspc31) and compared their results with PCR-enzyme-linked immunosorbent assay [32]. Real-time PCR in serum samples had better sensitivity. Surucuoglu et al. [33] used theTaqMan real time PCR technique which targeted the IS711, bcsp31 and per genes in patients with various clinical forms of brucellosis and compared the results of their method with other conventional methods using serum samples. Th IS711-based assay was the most sensitive, specific efficien and reproducible method to detect *Brucella* spp. Further reports have documented the specificity sensitivity and rapid results of real-time PCR [34,35].

Multiplex PCR

Th multiplex PCRs that have been developed the previous years have included also *Brucella* spp. with the firs report dating from 1994 [36]. Many reports can be found in the literature; the most interesting studies used multiplex PCR to simultaneous detect *Brucella* spp. and Mycobacterium Tuberculosis complex [37-39]. Th procedure targeted the *IS711*, *bcsp31* and *omp2a* genes for *Brucella* spp. and the *IS6110*, *senX3-regX3* and *cfp31* genes for *M. tuberculosis* complex. Since brucellosis is endemic in countries that are also endemic for tuberculosis [40], a rapid diagnostic test, especially in chronic and atypical cases is of great importance.

Future Developments

Researchers used the IS711 fragment to evaluate if PCR-ELISA is better than conventional PCR [41]. In this experimental study the use of PCR-ELISA proved to be of excellent sensitivity and specificity however the complexity of the method seems to be a drawback. Kim et al. [42] evaluated the application of single nucleotide polymorphisms (SNPs) by developing a new real-time PCR assay with a hybprobe from a specifi SNP to distinguish *B. abortus* from other *Brucella* species. The used the fba gene and in terms of sensitivity the real time PCR assay was equal to or higher than that of 16S rRNA PCR of previous studies [15]. However *B. abortus* is not the only pathogen, *B. melitensis* is far more common and identificatio by the use of SNPs has not been reported yet.

Conclusions

PCR based assays have been developed and used in various clinical and laboratory settings with promising results. Th sensitivity, specificit and diagnostic accuracy of the differen procedures are generally superior to standard diagnostic methods. Moreover the implementation of standardized real-time and multiplex PCR assays in clinical practice seems promising. Even though testing of PCR and real time PCR in large cohorts is lacking, molecular methods are used more and more in clinical practice.

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