

ORIGINAL ARTICLE

AQ1

Comparison of Diagnostic Methods in Hospitalized Patients With Brucellosis in Iran

AQ2

A.A. Amirzargar,* M. Hassibi,† P. Maleknejad,‡ H. Piri-Dougahe,‡ S. Jafari,† A.R. Soud Bakhsh,†
A. Davoodi,† M. Rasouli Nejad,† M. Haji Abdolbaghi,§ and Massoud Hajia, PhD||

AQ3

Abstract: Diagnosis of brucellosis is based on clinical symptoms and positive laboratory results. Because specificity of the serology tests is not satisfactory and sensitivity of the culture is low, polymerase chain reaction (PCR) can be an alternative method in making the final decision in some doubtful cases. In this study, results of standard tube agglutination, specific immunoglobulin (Ig) G and IgM by enzyme-linked immunosorbent assay, and culture were compared with PCR results in 45 patients with brucellosis referred to the Imam Khomeini hospital, Tehran, Iran. The BACTEC system was applied to isolate the organisms from blood samples, which were then identified by the urease, oxidase, H₂S production, and catalase tests. Primer pair was applied to amplify a 223-base-pair fragment of the *bcsp31* gene. Analysis of the results revealed that IgG test, IgM test, standard tube agglutination, culture, and PCR results were positive in 97.77%, 40%, 71.11%, 31.11%, and 48.88% of the cases, respectively.

(*Infect Dis Clin Pract* 2008;00:00–00)

Brucellosis is endemic in the Middle East and Mediterranean countries where it represents an important public health problem.¹ Clinical picture of the disease may show great variability. Patients with brucellosis are usually symptomatically treated by using different antibiotics of private clinics because of improper diagnosis. They will eventually be referred for hospitalization after getting several prescriptions. Therefore, its diagnosis requires laboratory confirmation. Culture and serology are the 2 most applied methods in diagnostic laboratories. Overall isolation rate is prolonged because of the low-level bacteremia and the slow growth of the organism. Serology, the next alternative method, has low specificity, particularly in patients living in endemic areas, in persons with a recent infection, and in those with suspected relapse.²

Today, molecular microbiology methods are increasingly needed in clinical laboratories, especially when dealing with hazardous pathogens, slow-growing organisms, and

viruses. Numerous polymerase chain reaction (PCR)-based assays have been developed for the identification of *Brucella* to improve diagnostic capabilities. Various target genes have been applied for genus-specific *Brucella* PCR such as *omp2*,³ 16S ribosomal RNA,⁴ IS7110,^{5–6} BCSP 31,⁷ and 16S to 23S ribosomal DNA interspace region,⁸ with different primer pair sets. Among these genes, primer pair targeting 223 base pair (bp) of the *bcsp31* gene encoding the 31-kDa protein is the most reported *Brucella* PCR protocol.⁹ This PCR set has been first reported by Baily et al¹⁰ after the comparison with different primer pair sets targeting the BCSP 31 gene. Sensitivity and specificity of this PCR set have been reported to be very high.¹⁰

The aim of this study was to compare the efficiency of PCR with routine diagnostic procedures in inpatients with brucellosis at the Imam Khomeini hospital.

MATERIALS AND METHODS

Patients

Forty-five patients with confirmed brucellosis based on clinical findings and laboratory results were included in this study at the Imam Khomeini referral hospital during the period from summer 2003 until summer 2005. All patients had a history of antibiotic therapy except 3 before being referred to the Imam Khomeini hospital. None of the patients had acute brucellosis; the clinical status of these patients was diagnosed as chronic brucellosis (approximately 80%) or relapse after the report of laboratory tests results. Fourteen of the patients were women, and the rest were men (age, 30–50 years old).

Brucella Strains and Samples

Brucella abortus strain 544 and *Brucella melitensis* serotype 1 (strain 16 M) were used for optimizing PCR. Ten milliliters of peripheral blood was drawn from each patient by the staff physicians. Five milliliters was inoculated in each standard aerobic BACTEC bottle at the patient's bedside. The rest was used for serology and PCR.

Considered Criteria for Patients With Brucellosis

Brucellosis was confirmed in our patients on the basis of signs and symptoms and positive laboratory results of serology, culture, and PCR or at least 1 of the applied tests.

Standard tube agglutination (STA)^{a,b}, enzyme-linked immunosorbent assay (ELISA)^c, immunoglobulin (Ig) M test, and IgG test were performed for all patients. The 2-mercaptoethanol test was not included in the applied

Departments of *Immunology, †Infectious Disease, ‡Microbiology, §Infectious Disease, Tehran Medical Sciences University, Tehran, and ||Research Center of Reference Laboratories of Iran, Iran.

This research was supported by funds from the Deputy of Research, Medical Sciences University of Tehran.

Address correspondence and reprint requests to Massoud Hajia, PhD, Research Center of Reference Laboratories of Iran. E-mail: massoudhajia@yahoo.com.

Copyright © 2008 by Lippincott Williams & Wilkins
ISSN: 1056-9103/08/0000-0000

AQ4