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ORIGINAL ARTICLE

Isolation of Brucella from blood culture of hospitalized brucellosis patients

Massoud Hajia, Mohamad Rahbar
Department of Microbiology, Research Center and Reference Laboratories of Iran

ABSTRACT

Background: Brucellosis is a zoonotic disease that is endemic in Iran. Appropriate and rapid diagnosis has a vital role in public health improvement. Low isolation rate of the organism has reported frequently in various reports. The present study was conducted to determine the isolation rate of organism in culture from collected specimens of hospitalized patients who were not under antibiotic therapy. Meanwhile, comparing the direct inoculation to biphasic media with lysis method was also determined.

Materials and methods: Twenty-five hospitalized brucellosis patients diagnosed on the basis of clinical manifestations and positive serologic tests were included. Blood samples were provided and cultured either as direct inoculation into biphasic media or lysis method by washing with distilled water before culture on solid media.

Results: Brucella was isolated in 4 samples (16%). Further studies revealed all these four cases to be *B. melitensis*. Washing method did not differ in isolation rate with direct inoculation; however, Brucella was isolated in a shorter period in washing method.

Conclusion: Higher isolation rate when compared with prior studies indicates an appropriate sampling time and technique, rapid inoculation to the media, and the lack of antibiotic therapy before sampling. Washing method has the preference of shorter isolation time to direct inoculation; however, it is faced with a higher risk of contamination.

Keywords: *Brucellosis, Laboratory diagnosis, Blood culture.*
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INTRODUCTION

Isolation of Brucella from clinical specimens is a matter of challenge in microbiologic laboratories. Brucellosis is endemic in Middle East and Mediterranean countries where it represents an important public health concern (1). Despite a significant reduction in incidence of brucellosis during the recent years, it is still a common infectious disease in rural areas (2). Clinical manifestations of the disease may show great variability, thus, laboratory confirmation is of utmost importance for definite diagnosis.

The organism is easily aerosolized. Culture and serology are two mostly applied methods in diagnostic laboratories. Overall isolation rate is low partly due to slow-growing of organism; however, patients are usually referred to hospital in Iran after different antibiotic therapies at private clinics. Serology, the next alternative method, entails low

Received: 4 October 2005 *Accepted*: 1 March 2006
Reprint or Correspondence: Massoud Hajia, MD.
Department of Microbiology, Research Center and Reference Laboratories of Iran.
E-mail: massoudhajia@yahoo.com

specificity particularly in patients living in endemic areas, subjects with a recent history of brucellosis and among those with suspected relapse. Different parameters are associated with false negative results (3). Various blood culture media have been introduced. Recent reports confirm higher isolation rate with rapid growth in a short time (4,5). Isolation rates are expected to be over 50% according to the previous reports (6). However, controversies exist in different studies (7).

PATIENTS and METHODS

Forty-one admitted patients were entered, among whom 25 had definite diagnosis of brucellosis according to the positive serologic criteria and clinical symptoms. All these patients with proved brucellosis had positive serological tests with standard tube agglutination (STA), Coombs, and 2ME tests (over 1/80,1/40,1/80 respectively). These tests were repeated after a while in some uncertain cases to ensure of rising antibodies. None of the patients was under treatment before commencing antibiotic therapy. This test was applied and carried out on the basis of Razi protocol kit.

A 10 ml-blood specimen was obtained from hospitalized patients during fever period. Then, 5ml was inoculated immediately into biphasic media (provided from Bahar Afshan Co.). Liquid phase was slightly transferred on the solid phase in regular time for isolation. Isolated suspected colonies were identified with Oxidase and Urease tests. Meanwhile, the remaining 5ml was transferred to the laboratory for washing method.

Washing method: All activities were carried out under biosafety cabinet. All specimens were washed with double distilled water (DDW). Specimens were mixed with 5ml DDW and then centrifuged for 30 minutes. The pallet was then cultured on blood agar. Oxidase and Urease tests were applied for isolated colonies.

RESULTS

Brucellosis was confirmed in 25 patients of 41 studied cases on the basis of the serologic results. Brucella was isolated in four (16%) cases (after 8, 11, 12 and 21 days on culture). Biochemical tests revealed all these four cases as *B. melitensis* (figure 1). The age distribution of patients is shown in table 1.

Table 1. *Age distribution of brucellosis patients*

Age group (year)	Male	Female	Total
0-9	0	1	1
10-19	2	5	7
20-29	2	1	3
30-39	1	1	2
40-49	5	1	6
Over 50	4	2	6
Total	14	11	25

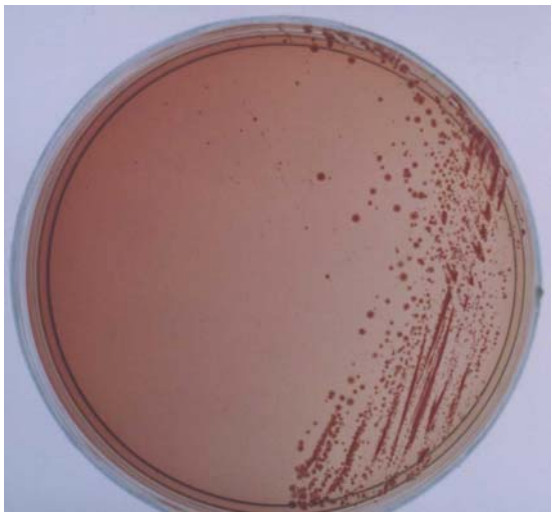

Figure 1. *Brucella colony in serum dextrose agar*

Having compared the two methods, it was confirmed that all positive cases were the same; however, washing method was faced with contamination. We detected two false positive cases that were misinterpreted in the direct inoculation method. Differential tests proved these two organisms were diphterioid and non-fermentative gram negative bacilli.

DISCUSSION

In our study, Brucella was isolated in 4 blood specimens of 25 approved brucellosis patients (16%), which is obviously higher than previous reports (8). These results confirm an appropriate sampling time. Nevertheless, isolation rate could be increased if more than one sample could be obtained from patients.

Washing method can theoretically influence the release of Brucella from polymorphonuclear (PMN) and take out any antibiotic materials presented in the blood sample; however, the present study failed to show its preference to direct inoculation method. Furthermore, it was afflicted with contamination.

To our knowledge, few studies have addressed isolation of Brucella and parameters influencing culture sensitivity in Iran. Diagnosis of brucellosis is based on the symptoms and serology results because of the low sensitivity of the culture method. Maghsoodi reported an isolation rate of 26.4% (29 out of 110 cases) that is relatively higher than our result (9). This discrepancy could be partly explained by different culture media. They used blood culture provided by Biomerux that contains CO_2 and are suitable for inoculation 10ml blood specimens; however, we used blood culture provided by Bahar-Afshan Company that is being used in many other laboratories as well. These media entail lesser specificities as compared with Biomerux media, thus, some *B. abortus* strains could not be isolated. On the other hand, Mansoori et al. could not isolate Brucella in hospitalized patients in Sina hospital in Kermanshah (10). Recently, Amirzargar et al. studied hospitalized brucellosis patients in Iman Khomeni Hospital in Tehran. They reported 14 isolated Brucella out of 45 cases despite using BACTEC system (11).

In summary, our isolation rate was obviously higher than those previously reported, although it is still very low when compared with expected rates. This could be partly explained by small sample size and blood culture type. Furthermore, low sensitivity of the culture may be due to clinical status of the disease in our patients.

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