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Improvement Isolation of *Brucella* spp from Blood Cultures of Suspected Brucellosis Patients using BACTEC 9120

G. Angoti[1], A. Karimi[2], F. Fallah[2], L. Gachkar[3], M. Rahbar[4,5]* and M. Hajizadeh[6]

[1]Department of Microbiology Shahid Beheshti, University of Medical Sciences, Tehran, Iran.
[2]Department of Microbiology, Pediatric Infectious Research Center (PIRC) of Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[3]Department of Pediatric, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[4]Department of Microbiology, Iranian Reference Health Laboratory, Ministry of Health and Medical Education, Tehran, Iran.
[5]Antimicrobial Resistance Center, Tehran University of Medical Sciences, Tehran, Iran.
[6]Department of Laboratory, Imam Reza Hospital, Tabriz, Iran.

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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 BACTEC(9120) system from collected specimens of suspected brucellosis patients. In this diagnostic- descriptive study 81 brucellosis patients from 361 suspected brucellosis patients diagnosed on the basis of clinical manifestations and positive serologic tests (SAT,Coombs wright,2ME) were included. Blood samples were provided and cultured either as direct inoculation into BACTEC system. Brucellosis was confirmed in 81 patients of 361(%59 females and %41 males) studied cases on the basis of the serologic results (SAT,Coombs wright,2ME). Brucella spp. was isolated in 19 samples (23.4%). Higher isolation rate when compared with prior studies indicates an appropriate sampling time and technique, rapid inoculation to the media.

Key words: *Brucellosis,* BACTEC, SAT, Coombs wright, 2ME.

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 of our country[2]. Clinical manifestations of the disease may show great variability, thus, laboratory confirmation is of the most importance for definite diagnosis. The organism is easily aerosolized. Culture and serology are two mostly applied methods in

* To whom all correspondence should be addressed.
Tel.: +98-2166728112-13; Fax: +98-2166728121
E-mail: rahbar_reflab@yahoo.com

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[3]. Serology, the next alternative method, entails low 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[4]. Various blood culture media have been introduced. Recent reports confirm higher isolation rate with rapid growth in a short time[5,6]. Isolation rates are expected to be over 50% according to the previous reports[7]. However, controversies exist in different studies[8].

Patients and methods

In this diagnostic- descriptive study eighty-one admitted patients were entered, among whom all 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 (SAT), Coombs wright, and 2ME tests (over 1/80,1/20,1/80 respectively). SAT test and Coombs anti*Brucella* test were performed according to previously described techniques[9].

These tests were repeated after a while in some uncertain cases to ensure of rising antibodies

This test was applied and carried out on the basis of Pasteur Institute kit. A 5ml-blood specimen was obtained from suspected patients during fever period. Then, inoculated immediately into BACTEC (9120) system (Becton Dickinson, USA).If no growth was detected within the usual five day protocol, incubation was incubated for 21 days, and blind subcultures were plated on chocolate agar (Merck Germany) After 7, 15 and 21 days. These subcultures were incubated at 37°C in 5-10% CO2 atmosphere for three days. If growth appeared, the suspected colonies were identified by colony morphology, Gram staining, oxidase, catalase, urease tests.Serotyping of the bacteria was not performed. All activities were carried out under biosafety cabinet.

RESULTS

Brucellosis was confirmed in 81 patients of 361(%59 females and %41 males) studied cases on the basis of the serologic results. *Brucella* was isolated in 19 (23.4%) cases (after 2-5 days on culture). Biochemical tests revealed all these 19 cases as *Brucella* spp. (Fig. 1). The age distribution of patients is shown in (Fig. 2).

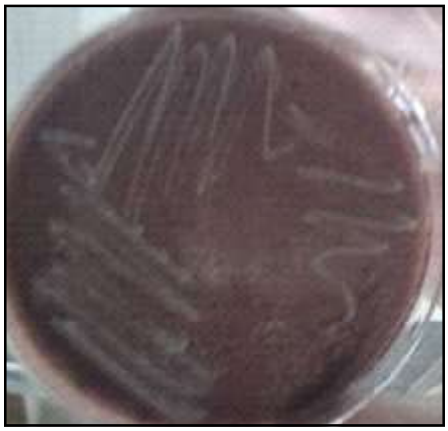

Fig. 1. Brucella colony in chocolate agar

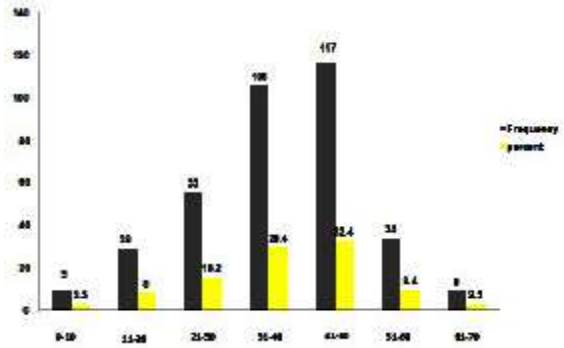

Fig. 2. The frequency & percentage based on age (years) in suspected patients to brucellosis

DISCUSSION

In our study, *Brucella* was isolated in 19 blood specimens of 81 approved brucellosis patients (23.4%), which is obviously higher than previous reports (10). These results confirm an appropriate sampling time. Nevertheless, isolation rate could be increased if more than one sample could be obtained from patients. The BACTEC method can theoretically influence the release of *Brucella* from polymorphonuclear (PMN) and neutralize any antibiotic materials presented in the blood sample. 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. In this study, we used BACTEC 9120 automated blood culture system and microorganisms were isolated from 81 cases (%23.4). In studies Surucuoglu that used the same our system, blood culture positivity rates were reported %36 from 18 cases [11]. The low rates of our results may be due to the fact that over %40 of the cases had chronic brucellosis or patients could have given incorrect information about antibiotic usage for various diseases. In studies Hajia *Brucella* was isolated in 4 blood specimens of 25 approved brucellosis patients (%16) [12].

The sensitivity of culture method could be enhanced by using bone marrow specimens but bone marrow aspiration remains an invasive and painful technique, therefore blood samples were preferred for culture. SAT test is important when the disease can not be detected by culture. SAT is widely used In most parts of the world for the diagnosis of brucellosis, because it is inexpensive, easy-to-perform, and rapid in comparison to culture[13]. In this study, SAT test was positive in 81 patients, and all chronic brucellosis could be diagnosed with serology.On the other hand, Mansoori et al. could not isolate *Brucella* in hospitalized patients in Sina hospital in Kermanshah[14].

Recently, Amirzargar *et al*. studied hospitalized brucellosis patients in Iman Khomeni Hospital in Tehran[15]. They reported 14 isolated Brucella out of 45 cases despite using BACTEC system, that it is still very low when compared with expected rates, although this isolation rate was obviously higher than our previously report[16].

In summary, this low isolation rate, 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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