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Case Study

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Rapid Identification of *Brucella melitensis* using BacT/Alert Blood Culture System: A Case Report

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A B S T R A C T

Brucella melitensis, a gram-negative coccobacillus, is the causative agent of the zoonotic disease brucellosis. Brucellosis can present with a variety of signs and symptoms hence the diagnosis becomes difficult. To ensure that the patient receives the appropriate treatment, laboratory testing is necessary to confirm the diagnosis. The advent of automated systems has made the identification of brucellosis easier. This facilitates the early initiation of appropriate antibiotics, thereby improving clinical outcome.

Introduction

Brucellosis is the most widespread zoonotic disease globally, with over 500,000 new cases reported each year (Mantur, 2008). It has been recognized as one of the 11 prioritized zoonotic infections in India (Stephen *et al.,* 2019). The symptoms of brucellosis in humans can differ and tend to be nonspecific. Therefore, it is essential to verify the diagnosis with laboratory tests to provide the patient with the correct treatment. Brucellosis can be diagnosed using culture methods, serological tests, and nucleic acid amplification assays. There is limited published data regarding the effectiveness of the BacT/Alert system in recovering *Brucella* spp (Yagupsky, 2019). Here we present a case report of Brucellosis which was identified by BacT/Alert after mean incubation time of 69.8 hrs.

Case Report

A 51 year old male patient, who is a Gardener by occupation presented with complaints of fever and headache for 1 month duration. Fever was gradual in onset, intermittent, low grade in the morning which progressively increased in the evening. Patient also had headache and pain over the neck for 1 month duration.

There was no history of vomiting, seizures or neck stiffness. He complained of multiple joint pain including both upper and lower limbs. Generalised fatiguability was present. Patient had been in Saudi Arabia for past one and half years and returned one week ago. He used to work there as a gardener and milk goats in farm. On examination, vitals were stable except temperature of 100.2°F. Systemic examination was within normal limit.

A paired blood culture was sent for the patient in view of suspected brucellosis and loaded in BacT/Alert automated blood culture system and the bottles flagged after 73.7 hrs and 65.9 hrs respectively.

It was subcultured on to 5% sheep blood agar which grew minute non-haemolytic colonies after 18 hours of incubation. In chocolate agar it appeared as white moist colonies. In culture Gram staining, it appeared as short gram-negative coccobacilli. Colony appearance in Chocolate agar and culture Gram stain is depicted in Figure 1.

The colony was catalase positive, oxidase positive, urea hydrolysed and non-motile by hanging drop motility method. MALDI TOF MS identified it as *Brucella* spp. with 99.9% confidence interval. Using gram negative identification panel in VITEK 2 system, it was identified as *Brucella melitensis.* The details of laboratory investigations done as a part of pyrexia of unknown origin workup is given in Table 1.

Echocardiography was done to rule out infective endocarditis. Contrast Enhanced Computed Tomography (CECT) of thorax and Abdomen was done to rule out any collections. Patient was empirically started on ceftriaxone 2g IV q24hr and C. doxycycline 100mg BD. After the blood culture report, rifampicin was added. Patient still had headache, which was not responding to analgesics. In view of persistent headache, CSF sample was sent for bacterial culture to rule out neurobrucellosis.

CSF culture turned out to be sterile, but the cell counts were high (100 cells with lymphocytic predominance). Non contrast Computed Tomography (NCCT) brain was normal. Fundus examination was normal.

With high clinical suspicion of neurobrucellosis, ceftriaxone dose was escalated to 2g IV q12h for 4 weeks. Patient clinically improved and was discharged with advice of T. Rifampicin 600mg OD x 3 months and C. Doxycycline 100mg BD x 3months.

Results and Discussion

Brucella spp. is a Gram-negative intracellular bacterium. Among the 12 recognized *Brucella* species, only four are pathogenic to humans: *Brucella melitensis*, *B. abortus, B. suis, and B. canis* (Kumari *et al.,* 2023). An Indian study reported 4.41% prevalence of brucellosis among the PUO cases (Pathak *et al.,* 2014).

Brucellosis in humans can be transmitted either directly or indirectly through inhaling infectious material from an affected animal or by consuming unprocessed animal products (Guzmán-Bracho *et al.,* 2020).

Incubation period is about 1 week to several months. The nonspecific symptoms include fever, chills, night sweats, and malaise, frequently accompanied by a severe headache and arthritis (Procop *et al.,* 2017). Less than 5% of patients have neurobrucellosis, which typically manifests as subacute or chronic meningoencephalitis. Usually more than 75% of times CSF is negative for culture, with blood culture positivity (Procop *et al.,* 2017).

In a study by Roiz *et al.,* (1998) mean time of detection using BacT/ALERT system was 67.8 hrs (Roiz *et al.,* 1998). Özkurt *et al.,* (2002) found no significant difference between BacT/Alert system and brucella broth culture with respect to growth time of the microorganism, but Brucella broth culture was more sensitive than the BacT/Alert system (Özkurt *et al.,* 2002). The mean time to detect *Brucella* spp. from blood specimens using the Castaneda method was reported to be 6.7 days (Baysallar *et al.,* 2006). In our study the mean time to detection was 69.8 hrs. In the early stages of the illness when serological testing is still negative or inconclusive BacTAlert system can identify *Brucella* spp (Christoforidou *et al.,* 2020).

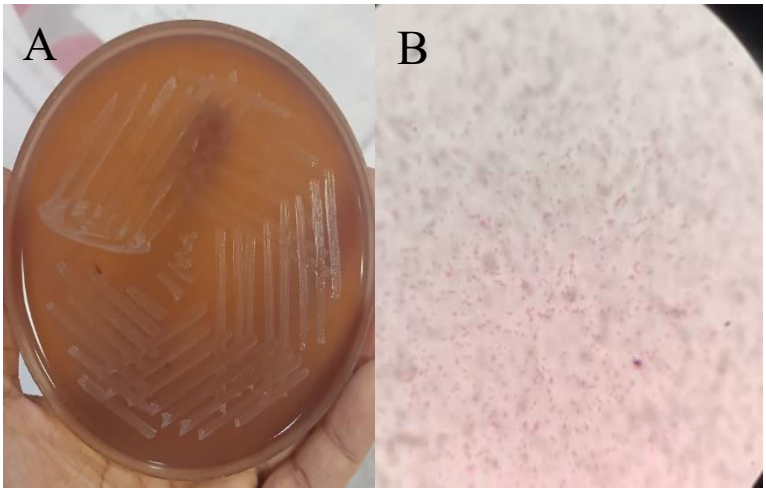
BacT/ALERT system &MALDI-TOF MS system give rapid and reliable identification of *Brucella melitiensis*. Certain commercially available biochemical test can misidentify *Brucella* spp. as Psychrobacter phenylpyruvicus or *Ochrobactrum anthropi.* Whereas VITEK MS (MALDI TOF MS) is not known to misidentify *Brucella melitensis* (Rudrik *et al.,* 2017). In our case, MALDI TOF has identified *Brucella* spp. with 99.9% confidence interval and VITEK 2 identified it as *Brucella melitensis.*

Early identification and prompt treatment is required to prevent complications of Brucellosis in which automated systems play a significant role. WHO recommends rifampin (600–900 mg/d) and doxycycline (100 mg twice daily) for 6 weeks for the treatment of Brucellosis in adults (Larry Jameson *et al.,* 2018). In case of significant neurological disease, treatment can be continued for 3-6 months along with ceftriaxone supplementation (Larry Jameson *et al.,* 2018). The same protocol was followed for our patient with which he has significantly improved.

Table.1 Laboratory investigations

Investigations	Value
Haemoglobin	15 g/dL
Total leukocyte count	8490/mm^3
Differential leukocyte count	$N_{45.6}L_{44.2}M_{9.5}$
MCV	83.8 fl
HCT	46.1%
Platelet count	2.70 L
Microbiological Investigations	**Report**
Urine culture	*Klebsiella aerogenes*
Brucella IgM ELISA	Positive (19.53 units)
HIV	Non-reactive
HBsAg	Negative
Anti HCV	Negative
Dengue IgM	Negative
Scrub typhus IgM	Negative
Chikungunya IgM	Negative

Figure.1 A. Chocolate agar showing white moist colonies of *B. melitensis* B. Culture Gram stain appearance of *B. melitensis*



Rapid identification and early treatment is necessary to reduce the morbidity and mortality associated with brucellosis. With the advent of automated systems identification of brucellosis become easier which helps in early initiation of appropriate antibiotics, thereby improving the clinical outcome.

Author Contributions

K. P. V. Hyma: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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