Evaluation of "rapid urease test performed on broth of blood culture bottles indicated positive by automated blood culture system" - as a tool for early diagnosis of brucellosis

Neha Patel, Urvesh Shah***

Abstract :

Brucellosis is a major zoonotic disease, the incidence of which is usually underestimated. The early diagnosis of human brucellosis continues to challenge clinicians because of its non-specific clinical features, slow growth rate in the blood culture, and the complexity of its serodiagnosis. Although the growth rate is slow, culture detection of circulating Brucella organisms remains a diagnostic cornerstone. The unique property of Brucella is to give urease test positive within 4 hours. So, we aimed our study to judge applicability of standard urease test done directly from blood culture bottles indicated positive by BACTEC 9050 for early diagnosis of brucellosis. **Materials & Methods:** The blood cultures indicated positive by BACTEC 9050 & having suspicion of Brucella infection were subjected to urease test. All the bottles were also sub-cultured over solid media for isolation & identification of bacteria. **Result & Discussion :** Out of total 50 bottles, 10 were found urease test positive within 4 hours of incubation (mean time 48 min) & all of these 10 bottles also revealed growth of Brucella spp. on subsequent subculture. Remaining 40 bottles, which were negative for urease test, revealed growth of other bacteria or didn't reveal any growth. The overall mean time for diagnosis of Brucellosis by blood culture & subsequent urease test was 74 hours with 100 % Positive predictive value. **Conclusion :** The rapid urease reaction was found to be the best and cost effective option to identify the Brucella spp. It can give positive reaction from the direct inoculation of blood from the blood culture bottle within 4 hours after indication of blood culture positivity by automated blood culture system; & thus it significantly reduces the time for preliminary identification of Brucella infection. This might also help to increase the rate of diagnosis for the Brucella spp.

Key words : Blood culture, Brucellosis, Rapid urease test

Introduction :

Brucellosis is a major zoonotic disease, the incidence of which is usually underestimated.[1] Human brucellosis is a debilitating disease that shows variety of symptoms, which makes it difficult to distinguish from other febrile conditions.[2] Mainly four species, Brucella abortus, Brucella canis, Brucella suis, and Brucella melitensis can infect humans and cause a systemic disease called brucellosis that is endemic in Mediterranean countries, the Middle East, Africa, and Latin America.[3] The prevalence of human brucellosis in India is 0.8% in Kashmir, 6.8 % in Varanasi, 8.5% in Gujarat, 11.51 % in Andhra Pradesh, 19.83% in Maharashtra and 26.6 % in Ludhiana.[4]

The early diagnosis of human brucellosis continues to challenge clinicians because of its non-specific clinical features, slow growth rate in the blood culture, and the complexity of its serodiagnosis.[5]

* Assistant Professor,
** Professor & Head, Department of Microbiology, GCS Medical College, Hospital & Research Centre, Ahmedabad, Gujarat, India
Correspondence :
Dr Urvesh Shah
E-mail : urveshv@yahoo.com

Although Brucella infections can be diagnosed with serological tests and nucleic acid amplification assays, culture detection of circulating Brucella organisms remains a diagnostic cornerstone.[6] The presence of antibodies does not always mean an active case of brucellosis, and therefore serological results must be interpreted with correlation of clinical and epidemiological data. The unequivocal proof of an active Brucella infection is the culture, and blood broth culture is the simplest and most often used procedure. Identification of Brucella spp. grown in blood culture bottle, is performed by using standard tests, including growth characteristics, Gram-staining, modified Ziehl—Neelsen stain, oxidase activity, urease activity, H_2S production (4 days) and sero-agglutination.[2] However, isolation & identification of Brucella spp. by blood culture is much time consuming & so remains under-diagnosed.

Most of the Brucella spp. give the urease test positive within 4 hours. [7,8] We aimed our study to judge applicability of standard urease test done directly from blood culture bottles indicated positive by BACTEC 9050 in suspected cases of brucellosis.

Materials and Methods :

In the present study, the blood cultures indicated positive by BACTEC 9050 & having suspicion of Brucella infection due to one of the following criteria were selected:

- Clinical suspicion of brucellosis
- Gram stain from positive culture bottles revealing gram negative or gram variable cocco-bacilli resembling Brucella spp. or no organism visualized on gram stain.
- The blood culture bottles, which were received from the patients of pyrexia of unknown origin & indicated positive by the automated instrument (BACTEC 9050) after 3 days of incubation.

All of these positive indicated bottles were subcultured on blood agar and nutrient agar. Broth from selected bottles as per the above criteria were also inoculated onto the Christensen's urease agar slant (M112, Himedia). All the sub-cultured media plates along with the urease slants were incubated at 37°C in the candle jar to provide CO_2. The inoculated urease slants were incubated at 37° C & observed for development of pink color at every 30 min upto a total period of 6 hours.

The inoculated plates were observed for demonstrable growth at every 24 hours till 5 days. The growth was further followed for identification by gram stain, oxidase test, catalase test & urease test from the colonies.

Result :

From the period of 2014 – 2018, total 50 blood culture bottles were selected as per the criteria laid down above. Gram stain examination of these positive growths revealed either gram negative coccobacilli or gram positive cocci (44), and some didn't show anything significant. [6]

After inoculation of urease agar slant, 10 slants showed pink color change within 4 hours of incubation (mean time 48 min). Out of them, 6 slants showed reaction within 30 minutes, 3 slants at 1 hour & only 1 slant after 2 hours of incubation. Others didn't show any color change even after 24 hours of incubation.

On further follow up, subculture from all the 10 bottles which were showing urease positive reaction, also revealed growth on blood agar plate after 24 to 48 hours of incubation. Colonies were small, smooth, transparent, low convex with entire edges and non hemolytic. Gram stain preparation from the colonies showed non capsulated, small, gram negative coccobacilli. All of them were subjected to oxidase test using oxidase disc (Himedia), which showed prompt purplish blue color change indicating positive reaction. Catalase test was performed using hydrogen peroxide,

which also gave positive reactions. Blood samples from these 10 patients were collected for serological tests (by agglutination) for brucellosis; all the 10 sera were also found positive for antibodies (with titer > 1:160)

Out of the other 40 bottles, which were negative for urease test, 26 showed growth of Co – agulase negative Staphylococcus, 6 were diphtheroids, 2 were B. subtilis, 1 was Salmonella typhi & 5 bottles didn't reveal any growth.

Discussion :

The clinical manifestations of human brucellosis are different and nonspecific; mimicking other infectious and noninfectious conditions; and affected patients required prolonged combination therapy with antibiotics. Therefore, laboratory confirmation of the diagnosis is of utmost importance for adequate case management. Although Brucella infections can be diagnosed with serological tests and nucleic acid amplification assays, culture detection of circulating Brucella organisms remains a diagnostic cornerstone.[6]

Brucellosis in human beings is rarely fatal, but can lead to severe debilitation and disability. Nevertheless, it is reported that approximately 2% of the untreated patients die of brucellosis. The disease may become chronic and persistent, or a granulomatous disease & can affect any organ system.[5]

The presence of antibodies does not always mean an active case of brucellosis and therefore serological results must be interpreted correlating with the signs and symptoms and epidemiology. Although in the last few years, PCR-based laboratory tests have been advised, they cannot be considered as a routine diagnostic tool yet, especially in developing countries where brucellosis is endemic.[2]

Blood cultures may also allow diagnosis of brucellosis in the acute period of the disease, when serological test results may give either negative results or exhibit borderline antibody titers.[9]

The majority of conventional Castaneda blood cultures for Brucella spp are positive between days 7 and 21 of incubation; and 2% are positive after day 27.[2] Joaquin Ruiz, Isabel Lorente found in their study, the Bactec system was better to grow the Brucella spp. in the BACTEC plus bottle (indicated positive within 2.5 to 5 days; average, 3.85 days).[10] Pablo Yagupsky found in the study, the BACTEC instrument detected 90 out of 97 (92.7%) positive cultures (of which 85 yielded B. melitensis and 12 were B. abortus isolates) within 5 days of incubation, and only 3 cultures (3.1%) became positive after the seventh day (2 on day 8 and 1 on day 9).[9] In the present study, blood culture bottles of all the 10 cases of confirmed Brucella infections, were indicated positive within 48 hours to 90 hours (mean time 73 hours) of incubation. This suggests use of automated methods can significantly reduce the time for blood culture positivity.

In study by Pablo Yagupsky, BACTEC broths containing Gram-negative coccobacilli and with no visible organisms were subcultured on urea slants and incubated in a CO_2-enriched atmosphere. Of the 44 Brucella isolates eventually recovered, 37 gave a positive urease reaction within 4 hours and the remaining were positive after overnight incubation. The urease test showed good specificity and only 2 isolates other than Brucellae (both Haemophilus influenzae) gave a delayed positive urease reaction.[9] Favorable results were also reported by Maleknejad et al. in an endemic area of Iran using a slight modification of the procedure of urease test.[7] Direct urease test on BACTEC blood culture bottles was also suggested by Michael Rich et al in their letter to editor. They found 100% of Brucella positive culture bottles giving urease test positive within 4 hours.[8]

In recent years, introduction of matrix-assisted laser desorption ionization-time of flight mass

spectrometry (MALDI-TOF) technology in the Clinical Microbiology laboratory has revolutionized the field of bacterial speciation, enabling precise and reproducible identification of isolates within minutes.[9] However, by looking towards the endemicity of Brucella in the developing countries, it is not possible every time to go for MALDI-TOF because of its lesser availability & cost of equipment. Thus, there is a need of simple and rapid identification technique which can be easily available at routine microbiology laboratory.

In the present study, all the 10 cases of Brucella infections had shown direct urease positive reaction within 4 hours of incubation. With inclusion of 73 hours of mean time to indicate a blood culture bottle positive by BACTEC, over all it took average less than 74 hours for diagnosis of Brucellosis with these modalities.

Conclusion :

The rapid urease reaction could be the best and cost effective option to identify the Brucella spp. It can give positive reaction from the direct blood culture bottle within 4 hours after indication of blood culture positivity by automated blood culture system; & thus it significantly reduces the time for preliminary identification of Brucella infection. This might also help to increase the rate of diagnosis of the Brucella spp., which might get undiagnosed due to delay in its growth on solid media & subsequent identification.

Acknowledgement :

The authors would like to acknowledge the active support of Department of Paediatrics & Department of Microbiology.

References :

1. Basappa G. et al ; Bacteremia is as unpredictable as clinical manifestations in human brucellosis; Int. J. of Inf. Dis. (2008) 12, 303-7.
2. Ducrotoya M et al ; Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control; Acta Tropica; 165 (2017), 179–193.
3. Nele Wellinghausen, KarstenNo¨ckler, Anja Sigge, Melanie Bartel, Andreas Essig, and Sven Poppert ;Rapid Detection of Brucella spp. in Blood Cultures by Fluorescence In Situ Hybridization; J. of Clin. Micro., May 2006, 1828–30.
4. Dakshayani P. Pandit, Padmakar T. Pandit; Human brucellosis: Are we neglecting an enemy at the backyard? Medical Journal of Dr. D.Y. Patil University; October-December 2013 , Vol 6,Issue 4, 350- 8.
5. Christopher S, Umapathy BL, Ravikumar KL ; Brucellosis: Review on the Recent Trends in Pathogenicity and Laboratory Diagnosis ; J. of Lab. Phy.; Jul-Dec 2010, Vol-2,Issue-2 , 55- 60.
6. Moshe Sagi, Lior Nesher, Pablo Yagupskyb; The Bactec FX Blood Culture System Detects Brucella melitensis Bacteremia in Adult Patients within the Routine 1-Week Incubation Period, J. of Clin. Micro., American society of Microbiology, 2017; Volume 55 (3), 942- 6.
7. P Maleknejad , FB Hashemi , B Fatollahzadeh , S Jafari ,H Peeri Dogaheh; Direct Urease Test and Acridine Orange Staining on Bactec Blood Culture for Rapid Presumptive Diagnosis of Brucellosis; Iranian J Publ Health, 2005, Vol. 34, No. 3, 52-5.
8. Michael Rich, Robert M. Bannatyne, and Ziad Memish ;Direct Urease Test on BACTEC Blood Cultures: Early Presumptive Diagnosis of Brucellosis in an Area of Endemicity; J. of Clin. Micro.;2000, 1706.
9. Pablo Yagupsky; Blood Cultures for the Diagnosis of Human Brucellosis; chapter http://dx.doi.org /10.5772/61143.
10. Joaquin Ruiz, Isabel Lorente, Jero´nimo Pe´rez, Encarnacio´n Simarro, and Leticia Marti´nez; Diagnosis of Brucellosis by Using Blood Cultures; J. of Clin. Micro., 1997, 2417–8.