

Three Cases of Brucellosis which Misidentified with Automated Bacterial Identification System

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ABSTRACT

Brucellosis can hold every organ and tissue, there is no specific clinical finding. In particular, the liver, bone marrow, spleen and lymph nodes as well as the organs of the lymphoreticular system, but more; heart, genito-urinary system organs, central nervous system can hold different organs and tissues, such as joints. Culture, serology, automated identification systems and polymerase chain reaction are used in the diagnosis. In this paper, three cases of brucellosis presented which misidentified with an automated bacteria identification system. The first case; 16-year-old female patient, pilonidal cyst, abdominal pain unspecified preliminary diagnoses, second case; 23-year-old female patient, bacterial meningitis, unspecified prediagnosis, third case; 15-year-old female patient: The bacteria were grown in blood and brain fluid samples which taken from patient. Misidentified as *Burkholderia gladioli* with automated system (Phoenix). Then we used traditional methods (catalase, oxidase, serology) for identification. The samples were found to be positive for oxidase, so we contacted to patients' clinicians for get additional test (Rose Bengal and Standard Tube Agglutination test (STAT). Rose bengal was found as positive, Tube agglutination was found to be 1/1280, 1/640 and 1/2560 positive, respectively. Bacterial identifications were used with conventional methods (oxidase, serology) and confirmed as *Brucella melitensis* by external laboratory with Vitec-2. Although proper treatment and eradication studies, brucellosis is still an endemic disease for our country. Brucellosis which misidentified by automated system. *B. melitensis* is the most common cause of disease in humans. These cases indicated to us deficiency of automated system. It should be update for identification of *Brucella spp.* and complete with conventional methods.

Keywords: Brucellosis; Automated bacteria identification system; Misidentified

INTRODUCTION

Brucellosis is one of the most common zoonotic diseases in the world and continues to be a public health problem and the cause of economic loss in developing countries. Brucellosis has no specific clinical manifestations because it can retain all organs and tissues. [1]. Although the organs of the liver, bone marrow, spleen, and lymph nodes, such as the lymphoreticular system, are more common; heart, genitourinary system organs, central nervous system can hold different organs and tissues such as joints [1]. Six species of *Brucella* genus have been identified. *B. melitensis* is the most common cause of the disease in humans. Culture, serology, automated identification systems and polymerase chain reaction are used for its diagnosis [2,3].

Case Report

Case 1

16-year-old female patient, preliminary diagnoses of pilonidal cyst, unspecified abdominal pain; Lab: Blood culture was regrown. Gram staining: Gram negative *Coccobacillus* was observed. We have used to automated bacterial identification system (Phoenix, Becton Dickinson, Sparks, Maryland, USA). The case was described as *Burkholderia gladioli*. It was seen oxydase (positive), additional test (Rose Bengal and Standard Tube Agglutination (STAT) was requested by contacting his clinician. Rose bengal: positive, STAT: 1/1280 positive. The

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culture sample was sent to the external laboratory. In which, it was described as *B. melitensis* with Vitec-2.

Case 2

23 year old female patient, bacterial meningitis, undefined pre-diagnosis; Lab: (CSF fluid-blood culture bottle was replicated. Gram staining: Gram negative *Cocobacillus* was detected. The sample was identified as *Burkholderia gladioli* with automated bacteria identification device. Rose bengal: positive. STAT: 1/640 positive. The culture sample was sent to the external laboratory in which it was defined as *B.melitensis* with Vitec-2.

Case 3

15 year-old female patient, preliminary diagnosis of Brucellosis; Lab: Blood culture was reproduction. Gram staining: Gram negative *Cocobacillus* was observed. We described to the example as *Burkholderia gladioli* by using Phoenix automated system. Oxidase was observed (positive), Rose bengal: positive, STAT: 1/2560 positive. The culture sample was sent to the external laboratory. It was described as *B.melitensis*. with Vitec-2.

DISCUSSION

Although proper treatment and eradication studies, brucellosis still remains an endemic disease for our country [3]. *B. melitensis* is the most common cause of the brucellosis in humans [1,2]. Automated systems are routinely used in most clinical laboratories for bacterium identification and antimicrobial susceptibility testing [3,4]. It should be noted that commercially available kits and *Brucella* species used in Gram negative bacteria identification can be misidentified [4,5]. However, in addition to gram negative identification card in Vitec 2 automated systems. It's reported that *B. melitensis* is defined by looking at the result of oxidase test [6]. However, Phoenix system identification and broth based antimicrobial susceptibility testing algorithms; uses fluorogenic and chromogenic substrates and a data processing application (Phoenix EpiCenter; Becton Dickinson AG). Unfortunately, *Brucella* spp. are not available in this database. For this reason, our isolates have identified as incorrectly. They have stand out as *Burkholderia gladioli*. There are very little data in the literature on this subject. This state may cause late or incorrect treatment of Brucellosis. Delays in the diagnosis of brucellosis can affect the prognosis of life-threatening complications (such as neurobrucellosis, endocarditis) [7]. Cekovska et al. [8]. According to the results of studies, Bact/Alert incubation system was used to isolate *Brucella* species from 16 blood cultures, using the automated Vitec 2 compact system; all strains were identified as *B melitensis*. BioMérieux Vitec 2 system and the Remel RapID NF Plus panel and the misdiagnosed *B suis* case as *Ochrobactrum anthrophia* were fatal with incorrect treatment [9]. The RapID NF Plus system and the strains identified as *Ochrobactrum anthrophia* and *Brucella* species were written using the rRNA sequence [10]. The rRNA results showed that all bacterial isolates were *Brucella* species, 100% reconciliation of known brucellae to the rRNA sequences and no homology to *Ochrobactrum anthrophia* sequences. The isolate was then serotyped as *Brucella suis* by

CDC [10]. Other authors reported the misidentification of *Brucella* species using automated identification systems. For example, *Brucella* spp. and *Ochrobactrum anthrophi* are genetically closely related genera, but despite their phylogenetic relationship these bacteria are very different respect to interaction with host cells and pharmacological treatments. Other studies reported that it must be careful when automated identification systems identify *O. anthrophi* and especially countries where brucellosis is endemic must be aware of the limitations of the automated microbiological system for *Brucella* identification [8-10]. Although *Burkholderia gladioli* is mainly known as a plant pathogen; it was reported as a human pathogen [11,12]. Our isolates have misidentified as *Burkholderia gladioli*. Experts agree that prompt identification of *Brucella* isolates is essential to provide appropriate treatment to patients and to control epidemiological outbreaks [13]. Misidentification of these highly infectious pathogens may lead to delays in diagnosis, but also to increased risks of accidental exposure for laboratory workers. MALDI-TOF mass spectrometer is reliable for *Brucella* identification to the genus level from culture plates and directly from blood culture bottles [13]. These cases showed that the deficiencies or limitations of automated systems should be well known. Consequently the database of automated bacterial identification system should update and complete with traditional methods for *Brucella* spp.

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