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Case Report

Brucella suis bacteremia misidentified as *Ochrobactrum anthropi* by the VITEK 2 system

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Abstract

Ochrobactrum and *Brucella* are genetically related genera of the family *Brucellaceae*, sharing 98.8% rRNA similarity. Because of their phenotypic similarity, *Ochrobactrum* can be miscoded as *Brucella* by automated identification systems. The misidentification on blood cultures (BCs) of *B. suis* as *O. anthropi* by the VITEK 2 system is herein described.

A 67-year-old male with a prosthetic mitral valve and fever was admitted with bacteremia due to a Gram-negative coccobacillus identified as *O. anthropi* by VITEK 2. The patient's fever persisted along with positive blood cultures despite specific antimicrobial treatment. Due to this adverse outcome, the patient was interrogated again and admitted having domestic swine. Serological tests were positive for acute brucellosis. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) of BC strains identified *B. suis* biovar 1. Timely identification of *Brucella* is essential for providing proper treatment to the patient and for advising safe handling of laboratory cultures in biological safety cabinets to prevent laboratory-acquired infection. Countries where brucellosis is endemic must be aware of this possibility.

Key words: *Brucella suis*; *Ochrobactrum anthropi*; zoonosis.

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Introduction

The family *Brucellaceae* comprises the genus *Brucella* and six further genera, namely *Crabtreella*, *Daeguia*, *Mycoplana*, *Ochrobactrum*, *Paenochrobactrum* and *Pseudochrobactrum*, phylogenetically members of the order *Rhizobiales* within the class *Alphaproteobacteria*. Organisms are aerobic, Gram-negative with rod-shaped morphology, and do not produce spores. *Brucella* species have been isolated from animals and humans, whereas the species belonging to the other genera of the family *Brucellaceae* have been predominantly isolated from environmental sources [1].

Brucellosis is the most widespread zoonosis of the world, with a prevalence of 10 per 100,000 population in endemic countries of South America, the Mediterranean basin, the Middle East, and the Arabian Gulf [2,3]. There are ten recognized species of *Brucella* with variable host preference, namely *B. abortus*, *B. suis*, *B. ovis*, *B. melitensis*, *B. canis*, *B. neotomae*, *B. pinnipedialis*, *B. ceti*, *B. microti*, and *B. inopinata* [4]. Some of the species are subdivided into biovars. Five *Brucella* species have been confirmed as human pathogens: *B. melitensis*, *B. abortus, B. suis, B. canis*, and recently *B. marina* [4]. Although *B. melitensis* is the most prevalent species in the world, in Argentina, one of the main species responsible for human brucellosis is *B. suis* [3], which consists of five biovars, of which 1, 2, and 3 are agents of porcine brucellosis. *B. suis* biovars 1 and 3 can cause severe human disease [5]. Porcine brucellosis is endemic in South America and Southeast Asia. *B. suis* biovar 1 infections have been described in the United States and Australia in humans who hunt and handle wild pigs [5,6].

Major routes of *Brucella* transmission to humans include consumption of unpasteurized dairy products and undercooked meat from infected animals, handling tissues (especially placenta and fetus) or fluids from infected animals without proper protection, and inhalation of *Brucella*-contaminated aerosols in a slaughterhouse or clinical laboratory. The infectious dose of *Brucella* is 10 to 100 organisms by the aerosol route [1]. Based to its easy aerosolization, *Brucella* species fall into the World Health Organization (WHO)'s Risk Group 3, *i.e.*, pathogens that pose a high risk to laboratory workers, requiring handling in

biological safety cabinets to prevent unintentional exposure [7]. Indeed, brucellosis is the most commonly reported laboratory-acquired infection [7], and people may be exposed to aerosols if they are near the culture while it is being manipulated [7].

O. anthropi is one of the closest *Brucella* relatives based on DNA, rRNA, and protein analyses [8,9]. Although both genera share phenotypic characteristics (*i.e.*, both genera are aerobic non-fermentative, catalase and urease positive Gram-negative rods) [5], they also have meaningful differences such as their motility and susceptibility to colistin. *Brucella* spp. are highly virulent intracellular pathogens whose isolation in humans provides irrefutable evidence of infection, whereas *Ochrobactrum spp.* are rhizosphere inhabitants that do not multiply within host cells and behave as opportunistic human pathogens [10] and only rarely cause human infection [11,12].

O. anthropi and *Brucella* share envelope molecules such as phosphatidylcholine and a lipopolysaccharide (LPS) with a lipid A carrying very long chain fatty acids; nevertheless, they differ in their lipid A-core chemical structure. *Brucella* has a reduced net negative charge on the core of its LPS, which confers this genera intrinsic resistance to bactericidal cationic peptides such as polymyxins [9]. *Ochrobactrum* is susceptible to polymyxins [10].

Epidemiological and clinical suspicion is critical for guiding microbiological diagnosis and establishing the significance of serology tests. Although polymerase chain reaction (PCR) is available, the initial diagnosis must be made by conventional identification methods [8].

Brucella spp. and *O. anthropi* may cause similar febrile diseases with non-specific clinical presentation [8,12-14]. Caution must be exerted when using biochemical test systems such as API 20NE [11] and automated systems such as RapID NF Plus system [1] and VITEK 2 for bacterial identification, because misidentification of these genera has been reported [5,15].

Case Report

On January 2013, a 67-year-old Caucasian male was admitted to the institution (a tertiary hospital with 120 beds) with a three-day history of fever, malaise, fatigue, and profuse sweats. He also complained of lower back pain. He was a native of Argentina and denied recent foreign travel and alcohol or drug abuse. His medical history was significant for mitral valve replacement in 2010. On clinical examination, his temperature was 39°C, pulse rate was 90 beats per minute, respiratory rate was 18 breaths per minute, and blood pressure was 130/60 mmHg. Pulmonary and abdominal examination revealed no abnormality. A mitral cardiac systolic murmur was present. Laboratory tests were remarkable for normocytic and normochromic anemia (3,460,000 cells/μL) and discrete leucopoenia (4.3×10^3 cells/μL) with a lymphocyte-to-neutrophil ratio within the normal range. Erythrocyte sedimentation rate (ESR) was 62, and C reactive protein (CRP) was 81.20 mg/dL. HIV serology was negative. Fasting plasma glucose levels were normal. Chest computed tomography was normal.

With clinical suspicion of prosthetic valve endocarditis (PVE), three sets (each set comprised of two BC bottles containing 10 mL of blood each) of BC (BACTEC aerobic bottles, BD Microbiology Systems, Franklin Lakes, USA) were taken, and the patient was empirically treated with ampicillin and gentamycin. After 72 hours of incubation, all BC (six aerobic bottles) were positive. Subculture on sheep's blood agar plates showed small grey bacterial colonies that grew slowly after 24 hours (Figure 1). The strain was a Gram-negative coccobacillus, catalase and oxidase positive, that grew in aerobiosis, and was identified as *O. anthropi* (BioMerieux, Marcy L'Etoile, France) VITEK 2 with a 99% match. Antibiotic susceptibility was performed using the disk diffusion method on Mueller-Hinton agar. Results were inferred as suggested for *Enterobacteriaceae* by the European Committee on Antimicrobial Susceptibility Testing [16]. The strain was susceptible to macrolides, fluoroquinolones, trimethoprim-sulfamethoxazole, and minocycline, and resistant to colistin and all beta-lactams. The patient's treatment was switched accordingly to intravenous ciprofloxacin (200 mg twice daily), clarithromycin (500 mg twice daily), and trimethoprim-sulfamethoxazole

Figure 1. Grey colonies on 5% sheep blood agar plate.

(160/800 thrice daily). After three days of this treatment, the patient remained febrile and bacteremic (*i.e.*, a set of BC taken at that time resulted positive for *O. anthropi*). After five days of treatment without improvement, a new set of BC resulted positive for *O. anthropi*. Transesophagic echocardiography (TEE) was negative for PVE. Although repeated TEE showed neither valvular vegetations nor abscesses, a clinical diagnosis of PVE was done based on Duke Criteria due to the presence of two major criteria, *i.e.*, persistently positive blood cultures (three sets of positive BC taken > 12 hours apart) and positive results of all of three separate sets of BC, and two minor clinical criteria (predisposing cardiac condition and fever > 38°C) [17].

Due to the adverse clinical outcome while on pathogen-directed treatment, the patient was interrogated again. When specifically asked about pets, he mentioned having swine at home (Figure 2). When asked about the health of the pigs, he stated that he had personally assisted a miscarriage of one of the animals two months before the initiation of symptoms without barrier protection, not even gloves. The patient's serum was tested for the presence of *Brucella* antibody, obtaining positive results for Rose-Bengal and buffered plate antigen tests. Acute *Brucella* infection was confirmed by further tests, namely the complement fixation test ≥ 1:80 (positive ≥ 1:4), standard tube agglutination test (STAT) of 3,200 UI/mL (positive ≥ 80 IU/mL), plate agglutination test > 800 UI and positive enzyme-linked immunosorbent assay (ELISA) IgG and IgM. Competitive enzyme immunoassay (CELISA) showed 68% inhibition (positive > 28% inhibition).

Antibiotic treatment was changed to oral doxycycline (100 mg twice daily) and intravenous gentamicin (5 mg/kg/day). The patient responded favourably, with progressive fever abatement and gradual normalization of ESR and CRP. After five days of this treatment, BCs were negative. Fourteen days later, treatment was switched to oral rifampicin (900 mg/day) and doxycycline (100 mg twice daily), which were continued for three months as suggested for *Brucella* PVE [5]. Patient outcome was successful and he did not require surgery.

In order to confirm diagnosis of brucellosis, BC bottles were sent to the national reference laboratory (Brucellosis Laboratory, National laboratories and Institutes of Health Administration, ANLIS, Dr Malbran CG, Buenos Aires, Argentina) where the isolates were identified as *B. suis* biovar 1 by PCR restriction fragment length polymorphism. After six weeks, the patient was discharged to complete treatment at home.

Figure 2. Poor conditions in which pigs were bred at the patient's home.

Figure 3. Patient´s home where pig farming is observed in precarious conditions.

Local sanitary authorities carried out an inspection of the patient's home for epidemiological purposes (Figure 3).

Conclusions

Ochrobactrum (from the Greek term *ochros*, meaning pale yellow because of the appearance of colonies on agar plates) and *Brucella* are genetically closely related genera of the family *Brucellaceae* within the class *Alphaproteobacteria* [8]. Despite their phylogenetic relationship, *Brucella* and *Ochrobactrum* are biologically different bacteria, especially with respect to interaction with host cells [9,18]. *Ochrobactrum* is a free-living environmental saprophyte with low virulence that does not replicate within human or animal cells and only recently and occasionally has been described as a cause of human disease [8,11,12,18], whereas *Brucella* is a well-recognized pathogen with the capacity for intracellular survival and replication that frequently affects animals and humans [8,19].

Ochrobactrum and *Brucella* also differ in their outer membrane; *Brucella* is highly permeable to hydrophobic substances whereas *Ochrobactrum* is not, leading to a different pattern of susceptibility. *Brucella* LPS core shows a charge reduction, which results in intrinsic resistance to polymyxins [10], whereas *O. anthropi* is susceptible to the latter. In the present report, it was notable that although the strain was identified as *O. anthropi*, it was resistant to colistin.

O. anthropi has been associated with occasional cases of endocarditis [12], bacteremia [18], and postoperative and nosocomial infections [20], mostly in immunocompromised patients [12]. The optimal treatment of *O. anthropi* infections is unknown [18].

Porcine brucellosis is caused by biovars 1, 2, or 3 of *B. suis*. Biovars 1 and 3 are pathogenic for humans. Because of its protean clinical picture, brucellosis may be clinically confused with other infectious or non-infectious diseases [3,5]. Accurate and rapid identification of *Brucella* spp. is necessary to provide appropriate treatment, especially when PVE is suspected [5], and additionally to prevent laboratory-acquired infection. The latter is only possible if *Brucella* is clinically suspected and consequently the cultures are managed in a biological safety cabinet. Also, identification of *Brucella* species and biovars is crucial for epidemiological purposes, because the isolation of *Brucella* from humans reflects its presence in the animal population and requires appropriate actions in order to prevent outbreaks [3]. Due to their phenotypic closeness, *O. anthropi* and *Brucella* spp. may be miscoded by automated identification systems, as occurred in the case we are reporting [8]. In this case, *B. suis* was misidentified by the VITEK 2 system. Previous reports have described erroneous identification of *Brucella* spp. by API 20NE, RapID NF Plus, and MicroScan systems [1,5,11,16].

Laboratories must be careful when automated identification systems identify *O. anthropi*. It must be remembered that common things occur commonly, and thus the isolation of an infrequent pathogen should raise questions. We highlight the importance of discussing activities including pet owning, hunting, travel, food consumption, occupation, and recreational activities when obtaining a history on patients with febrile illness. Countries where brucellosis is endemic must be aware of the limitations of the automated systems for *Brucella* identification. It is advisable to consider brucellosis when an automated microbiological system detects *Ochrobactrum* spp.

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