Mistaken Identity of *Brucella* Infection

Jing Yang, Xiao-qing Ren, Mei-ling Chu, Dong-ya Meng, Wen-cheng Xue

Clinical Laboratory, General Hospital of Shenyang Military Area Command, Shenyang, China

We read with interest the paper “Ribosomal RNA Sequence Analysis of *Brucella* Infection Misidentified as *Ochrobactrum anthropi* Infection” by Horvat and colleagues (1). The paper reported that a *Brucella* isolate was misidentified as *Ochrobactrum anthropi*. The 16S rRNA results indicated that the isolates were *Brucella* species, with 100% agreement to rRNA sequences of known brucellae and low homology to *Ochrobactrum anthropi* sequences.

Our laboratory has encountered such circumstances three times since December 2011. Strains were isolated from blood cultures of two patients suffering from fever without apparent cause and from a bone marrow culture of a patient with a left tibial lesion. They were initially identified as *Bordetella bronchiseptica* (1 case) and *Ochrobactrum anthropi* (2 cases) through the Vitek 2 Compact microbial identification system and Gram-negative (GN) identification card. As the first case (misidentified as *Bordetella bronchiseptica*) had brucellosis symptoms of splenomegaly, mildly elevated transaminase, typical undulant fever, and a significantly elevated lymphocyte level in his white blood cell count, 16S rRNA PCR amplification and sequencing were performed and results were analyzed using GenBank (accession numbers [ACBJ01000075.1](#), [ACEM01000005.1](#), [NC_013118.1](#), and [NC_009668.1](#)). The results indicated that the highest level of identity was to *Brucella abortus*, *Brucella melitensis*, and *Brucella microti* among the *Brucella* spp., as well as *Ochrobactrum anthropi* ATCC 49188 (query coverage, 100%; maximum identity, 99%). However, *Ochrobactrum anthropi* could be excluded based on a negative result observed in the semisolid-medium motility test and flagellar staining. The results suggest that the automated microbial identification system is unreliable, so two preserved strains identified as *Ochrobactrum anthropi* by the Vitek test were subcultured and reevaluated. 16S rRNA PCR amplification, sequencing, and alignment were performed. The results were similar to those for the first strain; i.e., highest identity was to several isolates within the *Brucella* spp. and *Ochrobactrum anthropi*. Here again, *Ochrobactrum anthropi* was excluded based on a negative result from the semisolid-medium motility test and flagellar staining. We also performed the test which utilized real-time PCR followed by high-resolution melting (HRM) curve analysis to identify the *Brucella* strains. They were identified as *Brucella melitensis*. All three strains were confirmed as *Brucella melitensis* by the China CDC, and the patients' sera were positive for *Brucella* antibody using the *Brucella* microagglutination test. These three stains were serotyped as biovar type I.

Brucella is a potential agent of bioterrorism and also a signifi-

cant pathogen causing laboratory-acquired infections (2, 3). However, misidentification of *Brucella* species by some commercial bacterial identification systems has been previously reported (4, 5). Because of these recurring misidentifications, more and more laboratories are now relying on molecular methods to identify *Brucella*. In addition to the report by Horvat and colleagues, studies by Gee et al. have shown that the *Brucella* species 16S rRNA consensus sequence, generated after 65 *Brucella* strains of 6 species were sequenced, shared 100% identity with 11 *Brucella* 16S rRNA gene sequences in GenBank, including *B. melitensis* strain 16M and *B. suis* strain 1330 (6). These results indicate that the 16S rRNA gene sequencing method is an efficient means of clinically identifying slow-growing Gram-negative bacilli, but we have serious concerns related to the complete absence of homology with *Ochrobactrum anthropi* compared with the 99% match to *Brucella* spp. or the *Ochrobactrum anthropi* strains (including the ATCC strain) reported here. Is this associated with the use of different databases (SmartGene versus GenBank)? We look forward to a reply from Horvat and colleagues so as to make better use of 16S rRNA sequence analysis to identify slow-growing bacteria such as *Brucella melitensis*.

REFERENCES

1. Horvat RT, El Atrouni W, Hammoud K, Hawkinson D, Cowden S. 2011. Ribosomal RNA sequence analysis of *Brucella* infection misidentified as *Ochrobactrum anthropi* infection. *J. Clin. Microbiol.* 49:1165–1168.
2. Chang MH, Glynn MK, Groseclose SL. 2003. Endemic, notifiable bioterrorism-related diseases, United States, 1992–1999. *Emerg. Infect. Dis.* 9:556–564.
3. Yagupsky P, Baron EJ. 2005. Laboratory exposures to brucellae and implications for bioterrorism. *Emerg. Infect. Dis.* 11:1180–1185.
4. Carrington M, Choe U, Ubillos S, Stanek D, Campbell M, Wansbrough L, Lee P, Churchwell G, Rosas K, Zaki SR, Drew C, Paddock CD, DeLeon-Carnes M, Guerra M, Hoffmaster AR, Tiller RV, De BK. 2012. Fatal case of brucellosis misdiagnosed in early stages of *Brucella suis* infection in a 46-year-old patient with Marfan syndrome. *J. Clin. Microbiol.* 50:2173–2175.
5. Elsaghir AA, James EA. 2003. Misidentification of *Brucella melitensis* as *Ochrobactrum anthropi* by API 20NE. *J. Med. Microbiol.* 52:441–442.
6. Gee JE, De BK, Levett PN, Whitney AM, Novak RT, Popovic T. 2004. Use of 16S rRNA gene sequencing for rapid confirmatory identification of *Brucella* isolates. *J. Clin. Microbiol.* 42:3649–3654.

Address correspondence to Wen-cheng Xue, wencheng_xue@hotmail.com.

For the author reply, see [doi:10.1128/JCM.00627-13](https://doi.org/10.1128/JCM.00627-13).

Copyright © 2013, American Society for Microbiology. All Rights Reserved.
[doi:10.1128/JCM.03222-12](https://doi.org/10.1128/JCM.03222-12)