Mistaken Identity of Brucella Infection

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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 brucelae 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 strains were serotyped as biovar type I.

Brucella is a potential 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