Case report

16S rRNA gene sequence analysis of a *Brucella melitensis* infection misidentified as *Bergeyella zoohelcum*

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Abstract
Misidentification of *Brucella* species from clinical specimens using commercial bacterial identification systems is a recurring problem. An isolate from a bacterimic patient was identified as *Bergeyella zoohelcum* by MicroScan Walk-Away (Siemens Healthcare Diagnostics Inc., West Sacramento, CA, USA) and as *Brucella melitensis* by Vitek 2 system (bioMérieux Inc., Durham, NC, USA). Because of this identification ambiguity by the two automated bacterial identification systems we performed 16S rRNA sequencing and serotyping of the isolate and confirmed it as a *Brucella* spp. Combining the sequence data with the Vitek 2 system data we conclude that the infection was caused by *B. melitensis*.

Key words: 16S rRNA sequencing; misidentification; *Brucella melitensis*; *Bergeyella zoohelcum*; automated bacterial identification systems

Introduction

Brucella is a potential agent of bioterrorism and also a significant pathogen causing laboratory-acquired infections [1,2]. It is therefore essential that all laboratories be able to identify this pathogen accurately and rapidly. The confirmative diagnosis of brucellosis is made by isolation of the pathogen from blood and other representative specimens. Automated blood culture systems such as BACTEC and BacT/Alert (bioMérieux, Durham, NC, USA) have significantly improved the time to isolation and rate of isolation of *Brucella* from clinical specimens [3]. However, identification of *Brucella* species using some commercial bacterial identification systems has not been consistent and misidentifications have occurred in the past [4]. *Brucella* species has been misidentified as *Moraxella phenylpyruvica* using the API 20NE system (bioMérieux, Marcy-I'Etoile, France) [5,6], *Ochrobacterium anthropi* by the API 20NE system [7] and RapID NF Plus system (Innovative Diagnostic Systems Inc., Atlanta, USA) [8], and as *Haemophilus influenzae* biotype IV and *Moraxella* species with use of MicroScan panels
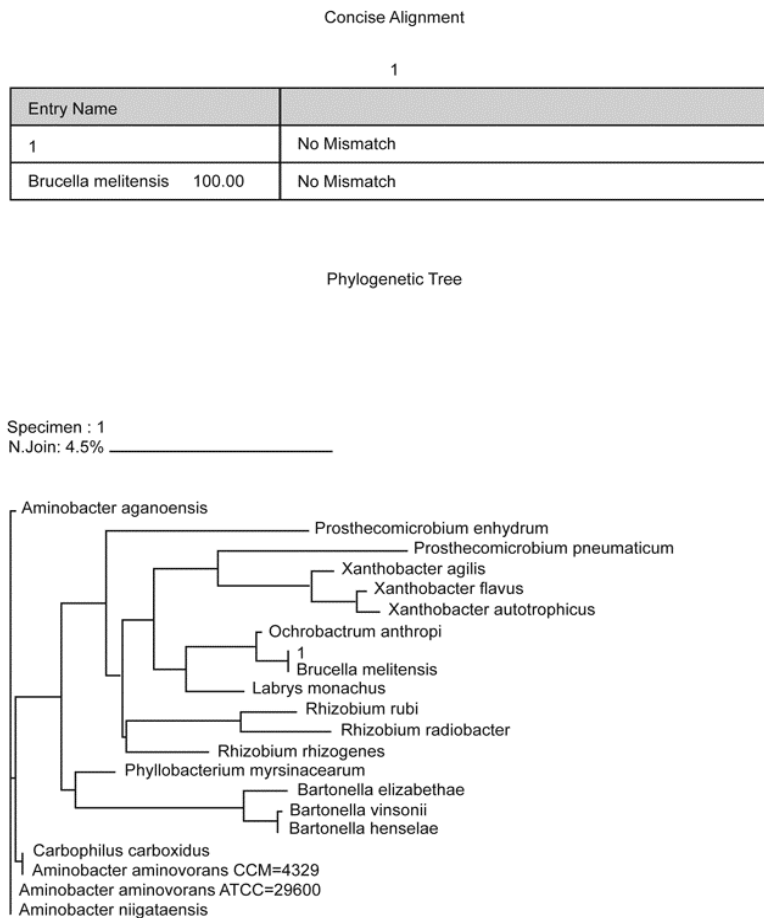
Siemens Healthcare Diagnostics Inc., West Sacramento, CA, USA) [7].

In this study, we describe misidentification of *Brucella melitensis* as *Bergeyella zoohelcum* by the MicroScan Walk-Away system (Siemens Healthcare Diagnostics Inc., West Sacramento, CA, USA). The isolate was correctly identified as *B. melitensis* by Vitek 2 and was subsequently confirmed as *B. melitensis* by 16S ribosomal RNA sequence analysis and serotyping.

Case report

A 35-year-old man was admitted to the intensive care unit of Al Qassimi hospital, a tertiary referral health care facility in Sharjah, United Arab Emirates, with working diagnosis of sepsis syndrome. The patient was critically ill having fever, hypotension, sinus bradycardia, bilateral pleural effusion and ascitis. Along with other laboratory investigations three sets of aerobic blood culture specimens were collected and sent to the microbiology laboratory. The blood cultures were positive for the presence of microorganisms by BacT/ALERT 3D system after 48 hours of incubation. The isolated organism was a

Figure. Phylogenetic tree of *Brucella melitensis*

Concise Alignment

Entry Name	
1	No Mismatch
Brucella melitensis 100.00	No Mismatch

Phylogenetic Tree

Specimen : 1
N.Join: 4.5%

Gram-negative cocobacillus that was catalase, oxidase and urease positive. The organism was identified as *B. zoohelcum* by the MicroScanWalk-Away system using MicroScan NegCombo Type 44 panel with a 64% probability. Due to the rareness of this pathogen, the isolate was re-identified using a Vitek 2 system (bioMérieux, Inc., Durham, NC, USA) Gram-negative card; in this case, identification was made as *B. melitensis* with a 99% probability. Confirmation of the identification was required to clear the diagnostic uncertainty and provide appropriate treatment to the patient.

Subsequently, 16S ribosomal RNA sequence analysis of the isolate was performed. Universal 16S rDNA primers (16SF: 5´-AGA GTT TGA TCC TGG CTC AG-3´ and 16SR: 5´- ACG GCT ACC TTG TTA CGA CTT-3´) [9] were used to amplify ribosomal DNA and sequencing was performed using

a BDT v3.1 sequencing kit (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). Both forward and reverse sequences of the 16S rRNA gene were analyzed using MicroSeqID v2.0 software (Applied Biosystems). A neighbor-joining tree populated with ten related species was created using the same software from the available bacterial DNA database (Figure). The organism was identified as *B. melitensis* with a match of 100%. The patient's serum was also positive for *Brucella* antibody using the *Brucella* microagglutination test.

Discussion

Accurate and rapid identification of *Brucella* spp. is necessary in providing appropriate treatment to affected individuals and taking appropriate public health measures to prevent outbreaks. The infection is often associated with laboratory-acquired

infections [10] and its use as a potential agent of bioterrorism is of concern [2]. In this case, the *Brucella* isolate was misidentified as *B. zoohelcum* by the MicroScan Walk-away, and was identified as *B. melitensis* by the Vitek 2 system. We believe such identification ambiguity can have several undesirable consequences. First, misidentification of *Brucella* species could result in accidental exposure among the laboratory personnel [11], in addition to having serious health consequences for the affected person in terms of inappropriate antimicrobial therapy, a longer hospital stay, chronicity, and relapse of the disease [12]. Secondly, this case reveals the uncertainties surrounding the use of bacterial identification systems for identifying *Brucella* during routine laboratory testing. Lastly, identification of a rare pathogen such as *B. zoohelcum* can create etiological perplexity and result in delay of appropriate antimicrobial therapy.

Misidentification of *Brucella* species by some commercial bacterial identification systems has been previously reported and might be due to several causes including relative biochemical inactivity of the pathogen, failure to incorporate identifying characteristics of *Brucella* species into their data bases, and lack of suitable panels for its accurate identification [4,13]. Because of these recurring identification concerns more and more laboratories are now relying on molecular methods to identify *Brucella* [8].

Molecular techniques using 16S rRNA sequence analysis are increasingly being used to identify *Brucella* to the species level and identify unusual *Brucella* strains from clinical specimens. Studies by Gee *et al.* have shown that the *Brucella* spp. 16S rRNA consensus sequence, generated after sequencing 65 *Brucella* strains from 6 species, had 100% identity with eleven *Brucella* 16S rRNA gene sequences from GenBank, including *B. melitensis* strain 16M and *B. suis* strain 1330 [14]. These results show that the 16S rRNA sequence obtained can be used to identify *Brucella* to the genus level, but cannot discriminate individual species.

We have utilized the MicroSeq v2.0 database (Applied Biosystems), a curated database that can be used to identify bacterial species based on sequence information. Current versions of validated sequence databases such as Microseq and RIDOM have been successfully used to identify dangerous bacterial pathogens including *Brucella* to the species level [15]. However various molecular approaches such as whole genome sequence comparisons, single nucleotide polymorphism (SNP) analysis of select genes [16], multilocus variable-number tandem-repeat (VNTR) analysis (MLVA), high-resolution melt (HRM) analysis, and differential real-time PCR assays have provided alternative approaches to detect and differentiate members of the genus *Brucella* to the species level. Overall, it has been demonstrated that the 16S rRNA in most *Brucella* species is highly conserved and, as a distinct marker of the species, enables rapid and accurate diagnosis of *Brucella* spp. infection without the need of routine diagnostic methods [15]. A combination of sequencing and either genetic or phenotypic testing can discriminate the isolate to species level if necessary.

Successful antimicrobial therapy of brucellosis depends on accurate and rapid diagnosis of the pathogen. In our case this is apparently demonstrated. The antimicrobial therapy of this patient began with intravenous amikacin per the hospital ICU protocol, and later ciprofloxacin was added to his regimen based on the blood culture report attributing *B. zoohelcum* sepsis. However, once the laboratory confirmation of the etiologic agent as *B. melitensis* was established, a combination regiment of rifampin plus doxycycline was recommended for a period of six weeks. He responded well and recovered.

Misidentification of *Brucella* species by some commercial bacterial identification systems can result in inappropriate treatment of the patient besides risking laboratory-acquired infections. While identification systems improve their diagnostic algorithm, molecular techniques wherever available can be used to confirm identification of the pathogen in cases of uncertainty, especially in countries where brucellosis is endemic.

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