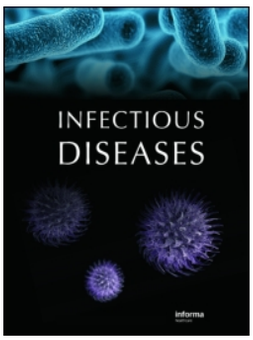

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LETTER TO THE EDITOR

Accuracy in identification of *Brucella melitensis* by the Vitek2 system: a report of two cases of misidentification

Recently, in this journal, the immunopathogenesis of brucellosis was reviewed [1]. *Brucella* has a marked capability to avoid the host resistance, implicating a high probability of progression and chronic development of the disease. In clinical handling of brucellosis, the authors emphasised the importance of diagnosis. Clinical diagnosis of the acute form of brucellosis requires a systematic anamnesis of the patient with special attention to identify the risk factors and to evaluate the symptoms properly. However, clinical diagnosis *per se* is not sufficient alone to sustain a reliable case inclusion, partly because initial symptoms are unspecific and common with other diseases (i.e. fever, malaise, arthralgia and headache) and partly because of the lack of exhaustive information from the patient to link with potential risk factors. In non-endemic settings, this last issue is of capital importance to redirect confirmation of unspecific clinical picture with laboratory diagnosis. Confirmatory diagnosis arises by positive isolation of the bacteria from blood cultures or other clinically relevant specimens (biopsies, bone marrow, fluids). To provide rapid and accurate patient care, laboratory hospitals use automated blood culture systems coupled with the identification of the isolated strain by systems as Vitek2, MicroScan WalkAway or MALDI-TOF. In case of identification of highly pathogenic bacteria, a series of measures are taken towards the patient and, when applicable, the risk of exposed laboratory personnel is evaluated. Therefore, it is very important that the bacterial identification is exact for presumptive treatment and biological risk management. The family of *Brucellaceae* consists of seven genera (*Brucella*, *Ochrobactrum*, *Crabtreella*, *Daeguia*, *Mycoplana*, *Paenochrobactrum*, and *Pseudochrobactrum*). With the Vitek2 system, *Brucella* spp. has been formerly misidentified as *Ochrobactrum anthropi*, and *Bordetella bronchiseptica* [2,3]. Here, we report two cases involving the misidentification of *Haematobacter massiliensis* and *Herbaspirillum frisingense* as *B. melitensis* using the Vitek 2 system. The two bacteria were correctly identified by exclusion with Bcsp131 *Brucella* spp.-specific PCR, the analysis of the 16S ribosomal gene sequence and the Bruker MALDI Biotyper IVD MSP Identification Standard Method 1.1.

Case report 1

On January 22, 2018 an infant of 25 months was brought by his mother to the emergency department of AZ Damiaan with repetitive febrile episodes (40 °C) and recurrent symptoms as weeping cough and facial rashes. Anamnesis disclosed a stay with the family in India the month before. Febrile seizures manifested since the infant was back in Belgium. At admission, the patient was pyretic (38.8 °C) without other particular additional clinical symptoms. Blood culture resulted positive after 2.71 days. The isolated strain was analysed by Vitek 2XL and identified as *B. melitensis* (score 99%). By Malditof Bruker (Library IVD 6763) 'No reliable identification' was found. With the MBT IVD library extension for highly pathogenic organisms as *Brucella* spp. it might be possible to get identification of *B. melitensis* by Malditof Bruker. However, at the time being that library was not yet available. The blood culture, the isolated strain and

a serum sample of the patient collected at admission were sent for confirmation to the National Reference Centre (NRC) for *Brucella* spp. Serology was negative with Rose Bengal, Wright's slow agglutination test (with or w/o EDTA) and IgG ELISA immunoassays; both hemoculture and strain were PCR negative and there was no isolation of the strain possible in *Brucella*-specific media. 16S rRNA PCR amplification, sequencing, and alignment analysis of the isolated strain was performed and resulted in *Haematobacter massiliensis* species.

Case report 2

A man of 39 years old presented at the outpatient clinic of Clinique Saint-Luc Bouge with general complains. Physical examination provided indication of a profuse inflammatory syndrome (CRP= 146 mg/L) and lumbar pain radiating to iliac fossa on the right side. Blood culture revealed the presence of a bacteraemia caused by a microorganism identified as *B. melitensis* by the Vitek system version 8.01 and as *Herbaspirillum frisingense* by the Bruker MALDI-TOF IVD (7171 spectra)(last update December 2017). At the NRC for *Brucella* spp. the presence of *Brucella* was excluded and further analyses confirmed the isolate being of the *Herbaspirillum* genus.

The use of automated systems for microbial identification in clinical laboratories provides routine support for prompt and early diagnosis in patients with consequent immediate care. Conversely, a number of reports highlight the possibility of incorrect identification of bacteria increasing the risk of incorrect patient management. Previously, *Brucella* spp. were commonly misidentified with bacteria belonging to the closely related genera of *Ochrobactrum* [2,3]. In this letter, we report the misidentification of *B. melitensis* with other two Gram-negative bacteria namely *H. massiliensis* and *H. frisingense*. *H. massiliensis* belongs to the *Rhizobiales* order as *B. melitensis*. Infection due to *H. massiliensis* has been associated with septicaemia and endocarditis [4,5]. *Herbaspirillum* spp. are nitrogen-fixing bacteria belonging to *Betaproteobacteria* another class as *B. melitensis*, the latter being of the *Alphaproteobacteria* class. *Herbaspirillum* spp. are recognised as pathogens or as endophytes of plants, and lately described as human pathogens in the context of cystic fibrosis, leukaemia, and bacteraemia [6,7]. In one case report, *Herbaspirillum* spp. was misidentified with *Ochrobactrum anthropi,* highlighting the high similar spectra of the two bacteria. Considering that *Ochrobactrum* is reportedly misidentified with *Brucella* spp., the three bacteria most probably share some common features at the analysis by these automated systems. As *Brucella* spp., infection with *H. massiliensis* and *Herbaspirillum* can cause bacteraemia and can be isolated from blood cultures.

A recent report from Canada describes the occurrence of 39 accidents on a two-year timeframe related to unidentified or misidentified pathogens of the biosafety class risk group 3 (RG3)/Security-Sensitive Biological Agents including *Burkholderia pseudomallei*, *Francisella tularensis* and *Brucella* species [8]. Resiliencies are to be found in the consulted clinical libraries when the incidents occurred or lack of suitable panels for *Brucella* identification. Misidentification of *Brucella* spp. or other slow-growing RG3 bacteria gives rise to concerns regarding both delayed patient management and post-exposure prohylaxis of potential exposed personnel. In Belgium, suspected strains can be sent to the NRC to be confirmed. As far as this algorithm is known and applied by general practitioners and clinical biologists, there are few risks for disease management, albeit yet this procedure implies a 1–2-day delay in appropriate patient treatment. Also, correct identification of *Brucella* at the species and biovar levels is essential for epidemiological surveillance when isolation unravels species of endemic nature. Isolation in humans reflects its presence in the animal population and requires appropriate actions in order to prevent outbreaks. Belgium is free of *B.melitensis* and bovine brucellosis, and the quasi totality of human cases are of imported nature from travels in endemic Mediterranean countries and/or countries where animal control measures are absent [9]. However, *B. suis* biovar 2, *B. ceti* and *B. pinnipedialis* reside in wildlife and clustered outbreaks in domestic animals due to *B. abortus* have occurred in 2012 and 2013 [10]. Correct identification of *Brucella* spp. therefore remains an essential question also in non-endemic countries.

As the databases of automated systems are to be filled with a large number of strains to identify *Brucella* spp. with uncommon pattern or strain with very similar spectra, companies developing automated identification systems should increase de number of strains to feed the accuracy of their libraries. As *Brucella* spp. are RG3 bacteria, support towards this goal can be found in specialised laboratories present in reference settings.

Disclosure statement

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