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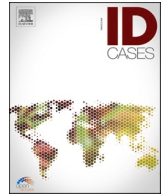
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Case report

Cutaneous Brucellosis unmasked as *Aureimonas altamirensis* in a wound cultureGeesje Roo-Brand^{a,*}, Theo A. Schuurs^b, Jan H. van Zeijl^b^a University Medical Center Groningen, Groningen, the Netherlands^b Certe Medical Microbiology Friesland and Noordoostpolder, Leeuwarden, the Netherlands

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ABSTRACT

Aureimonas altamirensis was isolated from a wound culture and initially misidentified as *Brucella melitensis* by the VITEK® 2 system. The VITEK-MS did not provide identification whereas the Bruker MALDI-ToF MS system and 16-S sequencing revealed a clear identification, which highlights the importance of inclusion of species in databases for accurate and fast identification of bacteria.

Introduction

Aureimonas altamirensis, formerly known as *Aurantimonas altamirensis*, the latter referring to Altamira Cave (Cantabria, Spain), was first isolated by Jurado et al. [1]. Since then this aerobic, gram-negative bacillus has been detected in human infections a number of times [2]. Here we present a case of *Aureimonas altamirensis* isolated from a wound culture that initially was misidentified as *Brucella melitensis* by the VITEK® 2 system (bioMérieux). The detection of a possible *Brucella* isolate causes concern in a clinical microbiology laboratory, because it can cause severe disease. Characteristics of the organism, including the fact that they are readily aerosolized and have a low infective dose, make it a frequently reported laboratory-acquired infection [3]. The revelation of *Brucella melitensis* as *Aureimonas altamirensis* was therefore a relief for the laboratory technicians and, in this case, was concluded to be of unclear clinical significance (colonization) for the patient.

Case

A 55 year old female patient without a significant medical history was diagnosed with carpal tunnel syndrome in December 2020 for which she underwent methylprednisolon/lidocaine injections. Three days after the injections she developed a swollen hand and several nodular lesions at the injection site. One month later the nodular lesions were unresolved however there was no clear diagnosis. We received a skin biopsy from one of the nodules for microbiological analysis. The

skin biopsy was cultured aerobically with 5% CO₂ on blood and chocolate agar (BD) and in serum broth at 35 °C for two days and anaerobically on Schaedler agar (ThermoFisher/Oxoid) and in Brucella broth (Mediaproduits, Groningen, the Netherlands) at 35 °C for 5 days. However the cultures yielded no growth. Histology showed granulomatous inflammation. Because of the atypical clinical presentation, lack of a clear diagnosis and a broad differential diagnosis, new biopsies were performed 2 months after the initial symptoms and were sent to our laboratory for further extensive research. Because of the origin of the patient and a recent travel history to the Dominican Republic, PCRs for *Leishmania*, *Mycobacterium leprae* tuberculous and non-tuberculous mycobacteria were performed and cultures for tuberculous and non-tuberculous mycobacteria were also carried out. All the PCRs and cultures were negative.

Microbial aerobic culture as described above did not yield growth, but the Brucella broth that was incubated anaerobically was routinely inoculated on two Schaedler agar plates after 5 days of incubation, of which one was placed under aerobic conditions (35 °C at 5% CO₂) and one under anaerobic conditions. After 24 h, the Schaedler agar under aerobic conditions showed growth of some remarkable yellowish colonies. The colonies were sub-cultured onto blood agar and MacConkey agar but only grew on the blood agar. Our first attempt of identification was performed by mass spectrometry with a VITEK® MS system (bioMérieux, database version 3.2), but this did not give a result (No ID). Identification with a VITEK® 2 system (bioMérieux) was performed which revealed *Brucella melitensis*. A Gram stain showed short gram

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negative rods and an oxidase test was positive, which is consistent for *Brucella*, however the yellowish colonies were inconsistent. The patient had no systemic complaints, and clinical findings were not typical, but there was a travel history to an enzootic area. The strain was sequenced for confirmation. In the meantime safety measures were taken in the laboratory, in case the isolate was confirmed as *Brucella*. 16S ribosomal DNA sequencing was performed using 16S forward (TGG AGA GTT TGA TCC TGG CTC AG) and reverse (TAC CGC GGC TGC TGG CAC) primers. A BLASTn search (NCBI) of the 16S rRNA gene sequence, using the non-redundant nucleotide open database, revealed distinctive results of 100% identity (298 nucleotides; coverage 100%) with *Aureimonas altamirensis* only.

Because of this unusual finding, confirmatory tests were performed in other external/reference laboratories. The National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands) performed a specific PCR for *Brucella melitensis*, which was negative. Furthermore, the strain was sent to a neighboring laboratory that uses the Bruker MALDI-ToF MS system (MBT Compass RUO Library version 9, revision F, 8468 MSPs). Analysis with the Bruker MALDI-ToF MS system identified the strain as *Aureimonas altamirensis* with a log score of 2.51, which is a very good score for confident species level identification. In the meantime susceptibility testing was performed using disk diffusion (ThermoFisher/Oxoid) on Mueller-Hinton agar with horse blood (BD). Zone diameters were Trimetoprim+sulfamethoxazol 6 mm, ciprofloxacin 29 mm, ceftazidim 36 mm, meropenem 36 mm, gentamycin 32 mm, Tobramycin 32 mm and Piperacillin 28 mm.

In this case there was a broad differential diagnosis in which, given the intervention and time course, an inflammatory reaction on injection of foreign body material was most likely, but could not be proven. Based on the histology of the skin biopsy, sarcoidosis was initially thought to be the most likely diagnosis. A microbiological cause came lower down on the list of possible differential diagnoses but had to be excluded. Culture as described above yielded *Aureimonas altamirensis*, but with no known cases of granulomatous skin inflammation related to *Aureimonas altamirensis* and histology findings more in-keeping with a non-infectious diagnosis, culture of this bacteria was considered to be environmental contamination. Unfortunately we were unable to investigate the injectable product for contamination with this micro-organism.

Discussion

Aureimonas altamirensis has been found in different clinical specimens including blood, ascites, pleural fluid, sputum, and in a corneal ulcer [2]. Remarkably, in one case of bacteremia with *Aureimonas altamirensis*, there was a clinical manifestation of cellulitis after a carpal tunnel release procedure [4]. The clinical skin presentation is not extensively described in this case but was different from the presentation in our patient. Moreover, they did not isolate *Aureimonas* from the

wound but *Staphylococcus aureus* instead, so it is unknown whether the *Aureimonas altamirensis* was of clinical significance for the cellulitis.

The misidentification of *Aureimonas altamirensis* by the VITEK-2 has been reported before, twice as *Brucella* [2,5] and once as *Acinetobacter lwoffii* [6]. The VITEK-MS did not provide identification because a reaction profile for *Aureimonas altamirensis* is lacking in the database. The Bruker MALDI-ToF MS gave a clear identification, which highlights the importance of the inclusion of genus/species in databases for accurate and fast identification of bacteria. Since *Brucella* is a highly contagious and pathogenic microorganism, suspicion for this species contains unnecessary commotion and safety measures at the laboratory and therefore it is undesirable that this misidentification occurs in the microbiology laboratory.

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CRediT authorship contribution statement

Geesje Roo-Brand: Conceptualization, Investigation, Writing-Original draft. **Theo Schuurs:** Writing – Reviewing and Editing. **Jan van Zeijl:** Conceptualization, Writing- Reviewing and Editing, supervision.

Declarations of interest

None.

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