urines, 1 endocervical swab, and 1 rectal swab), showing a rate of mutation of 23%.

A2059G point mutation was detected most often (5/9) (55.6%), followed by A2058G (2/9) (22.2%), A2058T (1/9) (11.1%) and A2058C (1/9) (11.1%). From those, 3 mutations were found among MSM (3/9), 3 in MSW (3/20) and 1 in a woman (1/10). Two episodes with non-available medical records harboured a mutation (one urethral swab and one first-void urine).

In 6 of the patients harbouring an AZM SNP (66.6%), this macrolide was used up to a month before in different clinical processes (*M.genitalium* infection, cellulitis episode and chronic bronchitis).

Our results broaden MRMs prevalence in Spain and are similar to those found in Barcelona[6], where the most prevalent mutation was A2059G (51.7%), followed by A2058G (41.4%), A2059C (3.4%) and A2058T (3.4%), with a total prevalence of 36%. In Gipuzkoa[7], MRM prevalence was of 16.3%, but the most common one, over the total prevalence, was A2058G (8%), followed by A2059G (7.2%) and A2059C (0.4%).

Moreover, MRMs were more frequent among MSM (3/9) (33.3%) compared to MSW or women (15% and 10%, respectively), which has been previously reported[6].

In conclusion, this study provides further evidence that macrolide resistance is highly prevalent in *genitalium* and supports the importance of MRMs detection in clinical laboratories to implement *resistance-guided sequential therapy*[3]. Additionally, the test of cure should be performed three weeks after antibiotic therapy to assess the treatment outcome[8].

Conflict of interest

The authors declare no conflicts of interest.

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Misidentification of *Brucella melitensis* as *Octrobactrum deajoenense* with MALDI-TOF MS: A report of three cases

Identificación errónea de Brucella melitensis *como* Octrobactrum deajoenense *con espectrometría de masas MALDI-TOF: informe de tres casos*

Dear Editor

Brucellosis is a zoonotic infection transmitted to humans from infected animals by ingestion of food products (such as unpasteurized dairy products) or by contact with tissues or fluids. It is the most common zoonosis worldwide and is an important public health problem in many developing countries.[1,2] *Brucella* species are oxidase positive, catalase positive, Gram-negative coccobacilli causing brucellosis. Four *Brucella* species (*B. abortus*, *B. melitensis*, *B. canis*, *B. suis*) are known to cause disease in humans, however, most human cases are caused by *B. melitensis*. The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF- MS) provides fast, easy to perform and cost-effective diagnosis in clinical microbiology laboratories.[3] Identification of *Brucella* spp. is not possible with MALDI-TOF MS, since this genus was not represented in the databases of the two main MALDI-TOF MS system manufacturers (such as bioMérieux and Bruker)[4,5] and this may cause the misdiagnosis of brucellosis. In this letter, we present three cases in order to draw attention to the misidentification of *Brucella melitensis* as *Ochrobactrum daejeonense* by MALDI-TOF MS (Bruker, Germany) in our clinical microbiology laboratory.

The first case was a 3-year-old female patient presenting with a complaint of intermittent fever and night sweats for a month. The patient had fever (38.5 °C) as well as abdominal pain and weight loss. She had a history of consuming raw dairy products. The second case was a 44-year-old male presenting with weakness, chills and loss of appetite. The patient, who was engaged in animal husbandry, had a draining wound on his foot one month before. The third case was a 55-year-old male patient presenting fever, arthralgia and night sweats. He was diagnosed with brucellosis two years before, as stated in his medical history. Blood samples of these patients were sent to Hacettepe University clinical microbiology laboratory with the pre-diagnosis of brucellosis. Aerobic and anaerobic blood cultures were incubated in the Bactec FX (Becton-Dickinson, USA) automated blood culture system. Single, tiny gramnegative coccobacilli were observed in routine Gram staining made from the blood culture bottle giving a positive signal (Fig. 1A). The samples were inoculated on sheep blood, MacConkey, and chocolate agar. Growth on blood and chocolate agars showed non-hemolytic, transparent, flat, small colonies with the absence of growth on MacConkey agar (Fig. 1B–D). All three isolates tested positive for

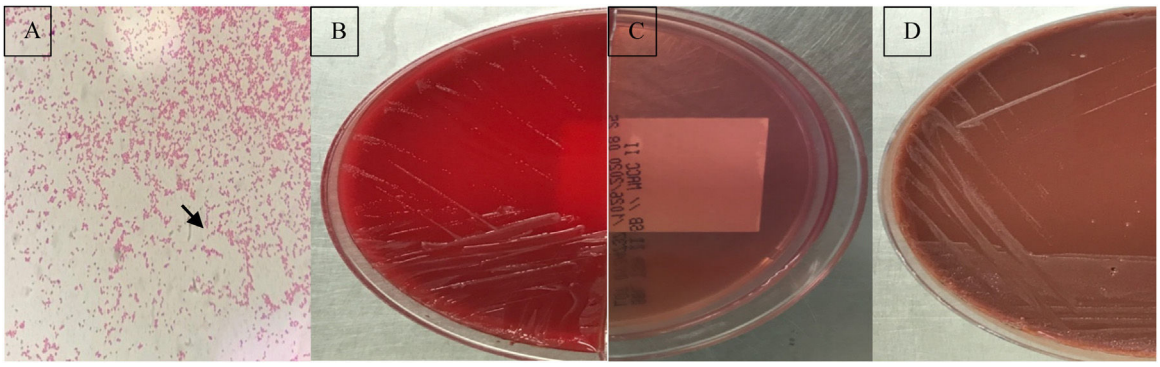

Fig. 1. (A) Gram stain of *Brucella melitensis*; (B) colony morphology of *Brucella melitensis* on blood agar; (C) absence of growth on MacConkey agar; (D) colony morphology of *Brucella melitensis* on chocolate agar.

oxidase, catalase and urease, and were identified as *Ochrobactrum daejeonense* by MALDI-TOF MS (Bruker MALDI Biotyper, USA) with score values of 1.7, indicating genus-level identification but then, the colonies tested positive in the slide agglutination test using polyvalent *Brucella* spp. antiserum. *Brucella* spp. antibody titers were 1/1280, as determined with the Metser Coombs Brucella Test (Metserlab Biological Products, Turkey) in serum samples taken simultaneously from all three cases. The positivity of serological tests for brucellosis was an important clue for the possibility of a misidentification.

The definitive identification was further confirmed by polymerase chain reaction-based amplification of 16S rRNA and sequencing. DNA was extracted using the MasterPure DNA purification kit following the manufacturer's recommendations, with a modification of lysis as described by Wu et al.[6] The amplification of the 1349-bp location of the 16S rRNA gene was performed using 27F 5′ AGAGTTTGATCMTGGCTCAG 3′ and 1492R 5′ TACGGYTACCTTGTTACGACTT primers. The cycles were: initial denaturation at 95 °C for 5 min, 40 cycles at 95 °C for 20 s, annealing at 57 °C for 45 s, extension 72 °C for 1 min, and final extension 72 °C for 5 min. The amplified products were run and viewed in a 1.5% agarose gel (Sigma, St. Louis, MO, USA). Sequencing was performed using BigDy Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, MA, USA). DNA sequences of the purified products were identified using ABI Prism 3700 Genetic Analyzer (AppliedBiosystems). The isolates were identified comparing the DNA reference isolates with data stored in the GenBank using the Basic Local Alignment Search Tool (BLAST version 2.0; http://www.ncbi.nlm.nih.gov/BLAST) program. A phylogenetic tree analysis was created using ClustalW MegAlign [https://www.ncbi.nlm.nih.gov/genbank/]. According to DNA sequence analysis 16S rRNA was 99% compatible with *Brucella melitensis*.

The family *Brucellaceae* consists of seven genera (*Brucella*, *Ochrobactrum*, *Crabtreella*, *Daeguia*, *Mycoplana*, *Paenochrobactrum*, and *Pseudochrobactrum*). *Brucella* and *Ochrobactrum* are closely related genera of the *Brucellaceae* family. *Brucella* spp. have been formerly misidentified as *Ochrobactrum antropi* with several automated systems.[7–10] MALDI-TOF MS is a reliable and rapid method for bacterial identification. Some databases used for this purpose lack reference profiles for *Brucella* species, due to its potential bioterrorism application. Bruker has a cooperation with the Centers for Disease Control (CDC) since 2016 (https://microbenet.cdc.gov/) so, people attempting the identification of bioterrorism microorganisms can be tracked. It would be useful for laboratory workers to have also this information as an alternative to the identification obtained directly from the mass spectrometer. When MALDI-TOF users obtain the identification "*Ochrobactrum* spp." and suspect misidentifications of *Brucella* spp., this suspicion can be confirmed using the CDC database, since *Brucella* species are still an important pathogen in wide areas around the world.

In this letter, three cases of *B. melitensis* were misidentified as *Ochrobactrum daejeonense* by MALDI-TOF MS in our clinical microbiology laboratory. The identification of the three isolates described in this letter was confirmed by PCR and sequencing of the 16S rRNA. There are several reasons why *Brucella* species are often misidentified in clinical laboratories. First, *Brucella* spp. infections are relatively rare, which leads to a lack of experience with this organism in clinical laboratories in some countries. Second, different bacterial automated identification systems are unable to correctly identify *Brucella* species, which misleads technical staff. Nevertheless, clinical microbiology laboratories in countries where brucellosis is endemic must be careful while using automated bacterial identification systems for identification of *Brucella* spp. as in our country.

In summary, accurate and rapid identification of *Brucella* species is most important for the early initiation of appropriate treatment. If there is a clinical suspicion of brucellosis, we suggest that the *Ochrobactrum daejeonense* or any other *Ochrobactrum* species results obtained with MALDI-TOF MS should be confirmed by additional tests such as biochemical, serological or molecular methods. These results should be evaluated with the correspondent clinical demonstration. Countries where brucellosis is endemic must be aware of the limitations of the MALDI-TOF MS for *Brucella* spp. identification. On the other hand, misidentification of *Brucella* spp. in the laboratory carries a high risk of laboratory-acquired infection due to aerosol generation and exposure among the laboratory personnel.[11] *Brucella* spp. should be handled under biosafety level 3 (BSL-3) conditions. Misidentification of *Brucella* spp. isolates as *Ochrobactrum* spp. can cause incorrect management of *Brucella* spp. cultures outside BSL3 laboratories.

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Competing interests

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Campylobacter fetus meningitis and subdural empyema☆

Meningitis y empiema subdural por Campylobacter fetus

Bacterial meningitis is a serious condition that requires early antibiotic therapy. Its most frequent aetiology is *Streptococcus pneumoniae* and *Neisseria meningitidis*. It is rare for the responsible pathogen to have an animal reservoir, as is the case of *Campylobacter fetus (C. fetus)*, whose reservoir is the digestive tract of cows and sheep, being a causal pathogen of zoonosis. Here we present a case of meningitis due to *C. fetus*.

The patient was a 59-year-old male smoker with no history of chronic alcohol consumption, hypertension or dyslipidaemia. He initially sought treatment for vomiting, diarrhoea and abdominal pain. After three days of dietary measures, the symptoms of fever (up to 40 °C), headache, prostration and dysarthria persisted, for which complementary tests were carried out, in which CRP 27.3 mg/dl, procalcitonin 0.4 ng/ml and 9,629 10^3/ug leukocytes with left shift (86.9% neutrophils) stood out. With the suspicion of infection of the central nervous system, a cranial CT scan was performed, which showed a left frontotemporoparietal hypodense subdural haematoma of 17 mm with deviation from the midline and another right frontotemporoparietal haematoma of 5 mm. With these findings, empirical antibiotic coverage was started with ceftriaxone, ampicillin, linezolid and acyclovir, and urgent surgery was performed with drainage of the collections. Initially, the clinical and radiological course was favourable, with decreased collections and no deviation from the midline, so a lumbar puncture was performed on the third day of antibiotic therapy, with yellowish-looking fluid coming out, with 13 predominantly monomorphonuclear leukocytes (86%), with glucose consumption (52 mg/dl in CSF, capillary glycaemia being 116) with proteinuria (304 mg/dl) and an outlet pressure of 13 mmHg.

One week after admission, the patient began to present a new neurological deterioration consisting of greater dysarthria. EEG with pathological tracing was performed, adjusting anticonvulsant treatment. Growth was detected in the subdural fluid samples sent in blood culture bottles (bioMérieux), so a Gram stain was performed directly from the bottle, this being highly suggestive of *Campylobacter* spp. Seeding was carried out on PVX (bioMérieux) and COS (bioMérieux) plates with incubation for 24 h and subsequent reincubation for another 24 h with no growth. It was also seeded on Campylosel agar (CAM-bioMérieux), incubating in a hood with GENbox microaer sachets for 48 h, observing growth of colonies, subsequently proceeding to identification with the Vitek MS system (bioMérieux). Sensitivity studies were carried out on Mueller-Hinton 2 agar +5% sheep blood (MHS-bioMérieux) with disc-plate in microaerophilia (in a hood with GENbox microbioMérieux sachets), being sensitive to carbapenems, so antibiotic therapy was modified to meropenem 2 g every 8 h, with the patient subsequently presenting a favourable clinical course. Antibiotic therapy was completed for four weeks and the anticonvulsant dose could be lowered until withdrawal. Prior to discharge, a brain MRI was performed, which showed a persistent left parietal subdural haematoma and discreet right meningeal thickening, without clinical repercussions, so no new drainage was performed. In subsequent follow-up, the patient remained asymptomatic.

C. fetus is a gram-negative bacillus found in the digestive tract of cattle and sheep, and can be transmitted after ingestion of contaminated food (such as milk or meat) or after direct contact with infected animals[1]. However, this contact with infected animals has only been identified in slightly more than half of the case[2].

Meningitis due to *C. fetus* is infrequent (0.02 per million inhabitants) and usually occurs in people with compromised immunity, generally with a history of alcoholism, liver disease, advanced age, diabetes mellitus, corticosteroid treatment or neoplasia[1].

The first published case dates back to 1960, and since then 37 cases of meningitis due to *C. fetus* have been documented (Table 1)[1–5], but only two case studies of empyema have been published[6,7]. Patients' average age is 50 years, and the majority are male (81%). As a risk factor, 27% have a history of alcoholism, 16% have diabetes mellitus, whilst 43% of cases are healthy patients. Although all the patients presented alterations in the biochemistry of the cerebrospinal fluid, in many cases the culture of the cerebrospinal fluid was negative (in up to 9 cases the only isolation was in blood), whilst the blood culture was positive in 81% of the cases. In 18%, the isolation was only in cerebrospinal fluid. Regarding the outcome, 72% were cured and there were three deaths among the published cases. There is no established treatment protocol. In the publications reviewed, many patients were treated with broad-

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