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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Alba Muñoz Santa*, Jesús Aramburu Arnuelos, Albert Bernet Sánchez, Alba Bellés Bellés

Sección Microbiología Hospital Universitari Arnau de Vilanova, Lleida, Spain

* Corresponding author.
E-mail address: amsanta.lleida.ics@gencat.cat (A. Muñoz Santa).

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

Identificación errónea de Brucella melitensis *como* Ochrobactrum 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 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

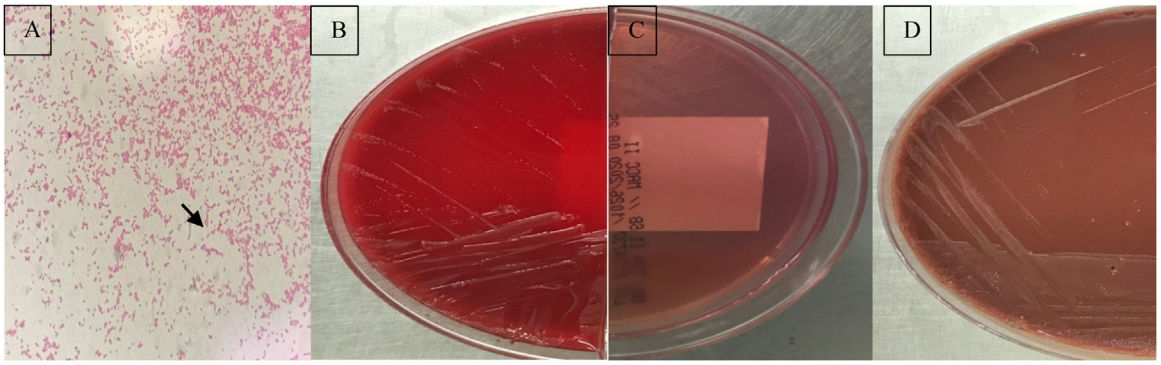

Fig. 1. (A) Gram stain of *Brucella melitensis*; (B) colony morphology of *Brucella melitensis* on blood agar; (C) absence of growth on MacConkey agar; (D) colony morphology of *Brucella melitensis* on chocolate agar.

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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Neşe Inal*, Gülşen Hazırolan

Faculty of Medicine, Department of Medical Microbiology, Hacettepe University, Ankara, Turkey

* Corresponding author.
E-mail address: nese-inal-108@hotmail.com (N. Inal).

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Meningitis y empiema subdural por *Campylobacter fetus*

Campylobacter fetus *meningitis and subdural empyema*

La meningitis bacteriana es una entidad grave que requiere antibioterapia precoz, su etiología más frecuente es *Streptococcus pneumoniae* y *Neisseria meningitidis*. Es infrecuente que el patógeno responsable tenga un reservorio animal, como es el caso de *Campylobacter fetus (C. fetus),* cuyo reservorio es el tracto digestivo de vacas y ovejas, siendo patógeno causal de zoonosis. A continuación presentamos un caso de meningitis por *C. fetus*.

Se trata de un varón de 59 años, fumador, sin historia de consumo crónico de alcohol, hipertenso y dislipémico. Consultó inicialmente por cuadro de vómitos, diarrea y dolor abdominal. Tras tres días de medidas dietéticas persistió la clínica asociando fiebre (de hasta 40 °C), cefalea, postración y disartria, por lo que se realizaron estudios complementarios, en los que destacaba PCR 27,3 mg/dl, procalcitonina 0,4ng/mL y 9629 10^3/ug leucocitos con desviación izquierda (86,9% neutrófilos). Con la sospecha de infección del sistema nervioso central se realizó TC craneal donde se describe colección subdural hipodensa frontoparietotemporal izquierda de 17 mm con desviación de la línea media y otra colección frontoparietotemporal derecha de 5 mm. Con estos hallazgos se inició cobertura antibiótica empírica con ceftriaxona, ampicilina, linezolid y aciclovir y se realizó intervención quirúrgica urgente con drenaje de las colecciones. Presentó inicialmente evolución clínica y radiológica favorables, con disminución de las colecciones y sin desviación de la línea media, por lo que se realizó, al 3.° día de antibioterapia, punción lumbar con salida de líquido de aspecto amarillento, con 13 leucocitos de predominio monomorfonuclear (86%) con consumo de glucosa (52 mg/dl en LCR, siendo la glucemia capilar 116) con proteinorraquia (304 mg/dl) y una presión de salida de 13 mmHg.

Tras una semana de ingreso comenzó a presentar de nuevo deterioro neurológico consistente en mayor disartria. Se realizó EEG con trazado patológico, ajustándose tratamiento anticomicial. En las muestras de líquido subdural enviadas en frascos de hemocultivos (bioMérieux) se detectó crecimiento, por lo que se realizó Gram directo del frasco, siendo éste muy sugestivo de *Campylobacter spp*. Se procedió a la siembra en PVX (bioMérieux) y COS (bioMérieux) con incubación durante 24 h y reincubación posterior otras 24 h con ausencia de crecimiento; además se sembró en Campylosel Agar (CAM-bioMérieux), incubándose en campana con sobres GENbox microaer durante 48 horas, observándose crecimiento de colonias, procediéndose posteriormente a la identificación con el sistema Vitek MS (bioMerieux). Se realizaron estudios de sensibilidad en Mueller-Hinton 2 agar +5% sangre de cordero (MHS- bioMérieux) con disco-placa en microaerofilia (en campana con sobres GENbox micro-bioMérieux), siendo sensible a carbapenems, por lo que se modificó antibioterapia a meropenem 2 g cada 8 horas, presentando posteriormente evolución clínica favorable. Se completó antibioterapia 4 semanas y se pudo descender la dosis de anticomicial hasta retirada. Previo al alta se realizó RMN cerebral donde persite colección subdural parietal izquierda y discreto engrosamiento meníngeo derecho, sin repercusión clínica por lo que no se realizó nuevo drenaje. En seguimiento posterior el paciente continuó asintomático.

C. fetus es un bacilo gramnegativo que se encuentra en el tracto digestivo de vacas y ovejas, pudiendo transmitirse tras la ingesta de alimentos contaminados (como leche o carne) o tras contacto directo con animales infectados[1]; sin embargo, este contacto con animales infectados solo se ha identificado en poco más de la mitad de los casos[2].

La meningitis por *C. fetus* es una entidad infrecuente (0,02 por millón de habitantes) que suele tener lugar en personas con inmunidad comprometida, siendo frecuente el antecedente de alcoholismo, enfermedad hepática, edad avanzada, diabetes mellitus, tratamiento corticoideo o neoplasia[1].

El primer caso publicado data de 1960, desde entonces se han documentado 37 casos de meningitis por *C. fetus* (tabla 1)[1–5], pero únicamente se han publicado dos casos de empiema[6,7]. La edad media es de 50 años, la mayoría son varones (81%). Como factor de riesgo, un 27% tiene historia de alcoholismo, un 16% de diabetes mellitus; siendo un 43% de casos pacientes sanos. Si bien todos los pacientes presentaban alteraciones en la bioquímica del líquido cefalorraquídeo, en muchos casos el cultivo del líquido cefalorraquídeo resultó negativo (hasta en 9 casos el único aislamiento fue en sangre), siendo en cambio positivo el hemocultivo en el 81% de los casos. En un 18% el aislamiento fue únicamente en líquido cefalorraquídeo. Con relación al desenlace, un 72% alcanzaron la curación, habiendo 3 muertes entre los casos publicados.

No hay un protocolo de tratamiento establecido. De las publicaciones revisadas, muchos fueron tratados con antibioterapia de amplio espectro o combinaciones, pero también se han reportado casos de pacientes curados tras monoterapia con ampicilina o amoxicilina[3]. Podríamos resaltar el hecho de que en un estudio multicéntrico se identificó una sensibilidad intermedia o resistencia del 12% a ampicilina, del 80% a cefotaxima y del 100% a eritromicina, así como el hecho de que hay reportados casos de cepas de *C. fetus* resistente a ceftriaxona, cefotaxima, y penicilina.