7. Nathanson M, Tisseron B, de Pontual L. Meningococcal vulvovaginitis in a prepubertal girl [Article in French]. Arch Pediatr. 2005;12:1732–3, http://dx.doi.org/10.1016/j.arcped.2005.09.012.

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Mycoplasma genitalium: Analysis of mutations associated with macrolide resistance in Lleida, Spain

Mycoplasma genitalium*: análisis de las mutaciones asociadas a la resistencia a macrólidos en Lleida, España*

Mycoplasma genitalium is a sexually transmitted pathogen responsible for 10-30% of non- gonococcal urethritis in men. In women, it is associated with cervicitis and complications such as pelvic inflammatory disease (PID) and possible infertility and poor obstetric outcomes[1,2].

The lack of cell wall in *M.genitalium* precludes the use of ß-lactams and limits its treatment choice to antibiotics such as tetracyclines (doxycycline-DOX), macrolides (azithromycin-AZM) and quinolones (moxifloxacin-MXF). Due to the decrease in cure rates with DOX, AZM is the recommended first-line treatment against *M.genitalium*[3,4]. However, since Jensen et al. in 2008 reported AZM treatment failure due to single-nucleotide polymorphism (SNP) mutation at positions 2058 and 2059 (*Escherichia coli* numbering) in region V of the 23S rRNA gene[5], the implementation of macrolide-resistance mutations (MRMs) assays has become of importance in order to minimize treatment failures. MXF is the second-line treatment recommended in such cases[3,4].

To date, limited data has been published regarding the prevalence of AZM resistance-associated mutations in Spain[6,7], so the objective of this study is to report the mutations found in the sanitary region of the province of Lleida between May 2019 and January 2021.

During this time, a total of 2288 specimens were tested for *M.genitalium*. DNA of the specimens was extracted using EZ1 or QIASymphony equipment (QIAGEN®), and real-time PCR screening was performed using the AllplexTM STI-7 V1-1 kit (Seegene®). Positive *M.genitalium* specimens were tested for MRMs with the Allplex MG&AziR (Seegene®) assay, which consists in a multiplex qPCR for detection of *M.genitalium* and six AZM SNPs (A2058G, A2058T, A2058C, A2059G, A2059T, A2059C). Both techniques were run on the CFX96 qPCR instrument (Bio-Rad®).

Of the 2288 of specimens, 46 samples from 46 patients (36 men and 10 women), consisting of 19 urethral swabs (41.3%), 14 first-void urines (30.4%), 10 endocervical swabs (21.7%) and 3 rectal swabs (6.5%), tested positive for *M. genitalium*, representing 2.1% of prevalence.

The request of sexually transmitted diseases (STD) testing of these samples were mainly urethritis (27) (58.8%), but also asymptomatic screening in high risk contacts (6) (13%), cervicitis (6) (13%), PID (4) (8.7%) and HIV pre-exposure prophylaxis (PrEP) (3) (6.5%).

The demographic and clinical data of the 39 infection episodes are described and classified by the sexual orientation in Table 1. Among them, 20 were men who have sex with women (MSW) (51.3%), 9 were men who had sex with men (MSM) (23.1%), and 10 were women (25.6%). Seven medical records, all from men, were not available.

Considering coinfections with other STDs, 9 *M.genitalium* cases (19.6%) were in coinfection with either *Ureaplasma urealyticum* (UU) (8.1%), *Chlamydia trachomatis* (CT) (6.9%), *Neisseria gonorrhoeae* (NG) (2.3%) and *Mycoplasma hominis* (MH) (2.3%).

Regarding MRMs, 37 episodes were classified as "wild type"/non-mutated (WT), whereas 9 cases (8 men and 1 woman) carried an AZM resistance mutation (3 urethral swabs, 4 first-void urines, 1 endocervical swab, and 1 rectal swab), showing a rate of mutation of 23%.

Table 1
Demographic and clinical data of the positive *M. genitalium* episodes classified by the sexual orientation of patients.

Total no. = 39[a]	MSM (n = 9) 23.1% No. (%)	MSW (n = 20) 51.3% No. (%)	Women (n = 10) 25.6% No. (%)
Medical history			
HIV positive	2 (22.2)	–	–
Syphilis history	3 (33.3)	2 (10)	1 (10)
HIV and syphilis	2 (22.2)	–	–
Clinical findings[b]			
Total symptomatic	**4 (44.4)**	**17 (85)**	**10 (90)**
Urethritis	4 (44.4)	17 (85)	–
Cervicitis	–	–	6 (60)
PID	–	–	4 (44.4)
Total asymptomatic	**5 (55.5)**	**3 (15)**	–
HIV pre-exposure prophylaxis (PrEP)	3 (33.3)	–	–
High-risk contacts	2 (22.2)	3 (15)	–
Specimens[c]			
Urethral swab	4 (44.4)	11 (55)	–
First-void urine	3 (33.3)	8 (40)	–
Rectal swab	2 (22.2)	1 (5)	–
Endocervical swab	–	–	10 (100)
Macrolide resistance by specimen[d]			
23S rRNA mutant MG	**3 (33.3)**	**3 (15)**	**1 (10)**
Urethral swab	1 (11.1)	1 (33.3)	–
First-void urine	1 (11.1)	2 (66.7)	–
Rectal swab	1 (11.1)	–	–
WT MG	**6 (66.7)**	**17 (85)**	**9 (90)**
Urethral swab	4 (44.4)	11 (64.7)	–
First-void urine	1 (11.1)	5 (29.4)	–
Rectal swab	1 (11.1)	1 (5.9)	–
Total no. coinfection[e]			
CT	–	3	2
NG	1	–	–
MH	–	–	1
UU	2	1	2

[a] Medical records were missing in 7 patients. [b,c,d,e] comments are referred to these episodes.
[b] Six patients were tested for STD because of urethritis and one was because of a high-risk contact.
[c] Four samples were urethral swabs and 3 were first-void urines.
[d] Five samples were wild type (WT) (2 first-void urines and 3 urethral swabs) and two carried a MG mutation (1 first-void urine and 1 urethral swab).
[e] In four of these samples, MG was in coinfection with CT, UU, NG and both MH and UU.

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.

Bibliografía

1. Sethi S, Zaman K, Jain N. Mycoplasma genitalium infections: current treatment options and resistance issues. Infect Drug Resist. 2017;10:283–92.
2. Lis R, Rowhani- Rahbar A, Manhart LE. Mycoplasma genitalium infection and female reproductive tract disease: a meta-analysis. Clin Infect Dis. 2015;61:418–26.
3. Read TRH, Fairley CK, Murray GL, Jensen JS, Danielewski J, Worthington K, Doyle M, Mokany E, Tan L, Chow EPF, Garland SM, Bradshaw CS. Outcomes of Resistance-guided Sequential Treatment of Mycoplasma genitalium Infections: A Prospective Evaluation. Clin Infect Dis. 2019;68:554–60.
4. Touati A, Peuchant O, Jensen JS, Bébéar C, Pereyre S. Direct detection of macrolide resistance in Mycoplasma genitalium isolates from clinical specimens from France by use of real-time PCR and melting curve analysis. J Clin Microbiol. 2014;52:1549–55.
5. Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin treatment failure in Mycoplasma genitalium-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. Clin Infect Dis. 2008;47:1546–53.
6. Fernández-Huerta M, Vall M, Fernández-Naval C, Barberá MJ, Arando M, López L, Andreu A, Pumarola T, Serra-Pladevall J, Esperalba J, Espasa M. Mycoplasma genitalium macrolide resistance update: Rate among a 2016-2017 cohort of patients in Barcelona. Spain. Enferm Infecc Microbiol Clin. 2020 Mar;38:99–104.
7. Piñeiro L, Idigoras P, De la Caba I, López-Olaizola M, Cilla G. Tratamiento antibiótico dirigido en infecciones por Mycoplasma genitalium: análisis de mutaciones asociadas con resistencia a macrólidos y fluoroquinolonas. Enferm Infecc Microbiol Clin. 2019;37:394–7.
8. Jensen Js, Cusini M, Gomberg M, Moi H. 2016 European guideline on Mycoplasma genitalium infections. J Eur Acad Dermatol Venereol. 2016;30:1686–93.

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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 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