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## Azithromycin and moxifloxacin resistance determinants in *Mycoplasma genitalium* in Lleida, Spain

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### Article history

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### ABSTRACT

**Introduction.** *Mycoplasma genitalium* (MG) is a microorganism related to sexually transmitted infections. Antibiotic resistance of MG leads to an increase in treatment failure rates and the persistence of the infection. The aim of this study was to describe the most frequent mutations associated with azithromycin and moxifloxacin resistance in our geographical area.

**Material and methods.** A prospective study from May 2019 to May 2023 was performed. MG-positive samples were collected. Real-time PCRs (Allplex™ MG & AziR Assay and Allplex™ MG & MoxiR Assay, Seegene) were performed in MG positive samples to detect mutations in 23S rRNA V domain and *parC* gene.

**Results.** A 37.1% of samples presented resistance determinants to azithromycin and the most common mutation detected was A2059G (57.9%). Resistance to moxifloxacin was studied in 72 azithromycin-resistant samples and 36.1% showed mutations, being G248T the most prevalent (73.1%).

**Conclusions.** The resistance to different lines of treatment suggests the need for a targeted therapy and the performing of a test of cure afterwards.

**Keywords:** azithromycin; moxifloxacin; mutation; *Mycoplasma genitalium*; resistance

### Determinantes de resistencia a azitromicina y moxifloxacino en *Mycoplasma genitalium* en Lleida, España

### RESUMEN

**Introducción.** *Mycoplasma genitalium* (MG) es un microorganismo relacionado con las infecciones de transmisión sexual. La resistencia antibiótica del MG conduce a un aumento de las tasas de fracaso terapéutico y a la persistencia de la infección. El objetivo de este estudio fue describir las mutaciones más frecuentes asociadas a la resistencia a azitromicina y moxifloxacino en nuestra área geográfica.

**Material y métodos.** Estudio prospectivo desde mayo 2019 a mayo 2023 en el que se incluyeron todas las muestras positivas para MG (una por paciente). Se estudió la presencia de mutaciones en el dominio V del ARNr 23S y en el gen *parC* mediante PCR en tiempo real (Allplex™ MG & AziR Assay y Allplex™ MG & MoxiR Assay, Seegene).

**Resultados.** Un 37,1% de las muestras presentaron determinantes de resistencia a azitromicina y la mutación más común detectada fue A2059G (57,9%). La resistencia a moxifloxacino se estudió en 72 muestras resistentes a azitromicina y el 36,1% presentaron mutaciones, siendo G248T la más prevalente (73,1%).

**Conclusiones.** La resistencia a diferentes líneas de tratamiento sugiere la necesidad de una terapia dirigida y la realización de una prueba de curación posterior.

**Palabras clave:** azitromicina; moxifloxacino; mutación; *Mycoplasma genitalium*; resistencia

### INTRODUCTION

*Mycoplasma genitalium* (MG) is a microorganism related to sexually transmitted infections (STI). The main route of transmission is direct genital-genital mucosal contact [1,2].

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MG diagnosis has been improved in recent years. The incorporation of molecular techniques in clinical laboratories has replaced the common culture because of its low sensitivity [3,4]. However, MG is still a clinical challenge. MG is intrinsically resistant to  $\beta$ -lactams due to the lack of cell wall, and therefore the choice of an appropriate treatment is limited to macrolides, tetracyclines, and quinolones [5]. According to the Clinical Guidelines, azithromycin is the first line of treatment and its cure rate is approximately 85% in macrolide susceptible infections, while doxycycline has a cure rate between 30-40%. The increasing resistance rates of MG to these antibiotics has led to the use of quinolones, such as moxifloxacin, as an alternative in macrolide-resistant or complicated infections [6].

Knowledge of the main mechanisms involved in MG antibiotic resistance is essential. Azithromycin inhibits protein synthesis and its resistance is associated with mutations in the region V of the 23S rRNA gene, being the point mutations at positions 2058 and 2059 the most frequent ones [7]. Azithromycin-resistant MG (Azi-R-MG) has been reported worldwide in the last years [2,5,8-11]. On the other hand, mutations in the *parC* and/or *gyrA* are responsible for moxifloxacin-resistant MG (Moxi-R-MG) [12]. Novel technologies to detect these mutations which include real-time PCR are excellent options to characterize these mechanisms when sequencing is not available [11]. The evaluation of acquired moxifloxacin resistance has been conducted in several studies, but the sample size evaluated is still not sufficient [1-3,7].

The aim of this study was to describe the prevalence of MG mutations associated with azithromycin and moxifloxacin resistance in the sanitary region of Lleida, Spain.

## METHODS

A prospective study from May 2019 to May 2023 was performed at Hospital Universitari Arnau de Vilanova (Lleida, Spain), a referral tertiary hospital in northeast Spain covering an area of approximately 340,000 inhabitants.

MG-positive samples from patients with clinical suspicion of a STI, sexual partner diagnosed with a STI or as a screening test for pregnant women with less than 25 years old were collected. The specimens included urethral swabs, first-void urines, endocervical swabs and rectal swabs. Only one positive sample per patient was evaluated.

DNA was extracted using EZ1 or QIASymphony equipment (QIAGEN), and real-time PCR screening was performed on the CFX96 qPCR instrument (Bio-Rad) using the Allplex™ STI Essential Assay (Seegene) for the detection of MG and other pathogens causing STI (*Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*).

A multiplex qPCR assay (Allplex™ MG & AziR Assay, Seegene) that detects MG and the most frequent mutations associated with azithromycin resistance (A2058G, A2058T, A2058C, A2059G, A2059T, A2059C) was performed in MG-positive samples.

Samples from patients already studied (less than six weeks), asymptomatic and/or with different kind of specimens the same day were excluded from resistance study.

Azi-R-MG samples were also tested for moxifloxacin resistance determinants since December 2019. Thereby, a multiplex qPCR (Allplex™ MG & MoxiR Assay, Seegene), that detects MG and six frequent mutations in *parC* gene (A247C, G248A, G248T, G259A, G259C, G259T) was performed.

The medical records of Azi-R-MG patients were reviewed by collecting demographic data, antibiotic treatment prescribed, the ongoing coinfections with other pathogens causing STI (CT, NG, TV) and the performance and result of test of cure (TOC), described as a second PCR performed for the detection of MG at least 4 weeks after the end of the treatment.

## RESULTS

A total of 256 MG-positive samples were analyzed during the period of the study. MG was detected in 178 samples from men (69.5%) and 78 from women (30.5%). The median age of MG-positive patients was 27 years (range 17-69). Demographic and clinical characteristics of patients are summarized in Table 1.

The qPCR assay that detects the most frequent mutations associated with Azi-R-MG was performed on the 256 samples. Determinants of azithromycin resistance were detected in 95 samples (37.1%, 95/256) and 75.8% of them (72/95) were men. The most frequently detected mutation was A2059G (55/95), followed by A2058G (22/95), A2058T (10/95), A2059C (6/95) and A2058C (2/95).

Regarding moxifloxacin, the qPCR assay that detects quinolones resistance determinants was performed on 72 Azi-R-MG samples since December 2019. Mutations in *parC* were found in 26 samples (26/72, 36.1%). The most frequently detected mutation was G248T (19/26), followed by G248A (4/26), G259A (2/26) and G259T (1/26). No A247C or G259C mutations were detected.

Seventy two of the 95 Azi-R-MG samples were detected in men and 38 (52.8%) of them were men who had sex with men (MSM). A mutation related to Moxi-R-MG was detected in seven of these 38 MSM (18.4%).

Data about previous antibiotic treatment was available in 89 patients, 45 of whom (45/89, 50.6%) were treated with azithromycin before this episode.

The 7.4% (7/95) of Azi-R-MG cases presented co-infections with other pathogens causing STI: CT (n=6) and TV (n=1). The 22.1% (21/95) of patients with Azi-R-MG were HIV-positive (20 men and 1 woman). HIV-positive men were all MSM.

Data on TOC performance was available in 70 of the 95 Azi-R-MG patients. The 87.1% (61/70) showed a negative TOC (no detection of MG). In the case of the specimens with a Moxi-R-MG, data was available in 21 cases and 15 patients had a negative TOC (15/21, 71.4%). G248T mutation in *parC* gene was the most common mutation (n=4) in individuals with a positive TOC.

Total MG positive samples (n=256)		Men		Women		
		178	69.5%	78	30.5%	
AZI-R-MG	95/256	37.1%	72/178	40.4%	23/78	29.5%
MSM			38/72	52.8%		
HIV+	21/95	22.1%	20		1	
First void urine	50/95	52.6%	46		4	
Urethral swab	10/95	10.5%	10		0	
Rectal swab	16/95	16.8%	16		0	
Endocervical swab	19/95	20.0%	0		19	
<i>parC</i> mutations test performed (n=72)			51	70.8%	21	29.2%
MOXI-R-MG	26/72	36.1%	13/51	25.5%	13/21	61.9%

AZI-R-MG (Azithromycin-resistant-MG), MOXI-R-MG (Moxifloxacin-resistant-MG).

## DISCUSSION

In this study we described the mutations in MG that confer resistance to azithromycin or moxifloxacin. It is a continuation of a previous one performed between 2019–2021 in which Muñoz-Santa *et al* evaluated the prevalence of MG and mutations related to azithromycin resistance in our area [5]. The main mutation detected in Azi-R-MG in the present study has been A2059G (55/95, 57.9%), which was the most frequent in the fore-mentioned report (55.6%). Our results are similar to those performed in other Spanish areas: a study performed in Tenerife (Canary Islands, Spain) found that 17 out of 28 samples have the A2059G mutation [11], and in a report from the south of Spain, the same mutation was described in the 50% of Azi-R-MG samples [9].

The increasing azithromycin resistance in different geographical areas points us towards the study of moxifloxacin resistance, the second line of treatment according to Clinical Guidelines [6]. In our study, moxifloxacin resistance was assessed in samples that were already resistant to azithromycin. The main *parC* mutation detected was G248T (19/26), which agrees with previously reports [3,7,10]. In our study, we only evaluated the *parC* gene, although some studies reported that resistance to moxifloxacin is also due to mutations in *gyrA* gene [11,14,15].

We reported 9/70 (12.9%) MG cases treated with azithromycin with treatment failure. A2059G was the mutation detected in 7 of these patients. It is unclear if there is a connection, or it is just because it is the most frequent mutation detected in different studies [3,5,7]. That is not the case with the *parC* G248T mutation, which is the main mutation described in association with moxifloxacin treatment failure [10,14]. This mutation was found in 4 (66.7%) of our 6 Moxi-R-MG samples with a positive TOC. However, more studies are needed to conclude that the mutations detected are responsible for treatment failure.

The presence of co-infections with others pathogens causing STI means the treatment prescribed was not restricted to MG. Five patients with co-infections (5/7, 71.4%) were negative for the TOC performed. The resolution of the infection could be explained by the involvement of different antibiotic treatments, but it would be necessary to consider that we have a limited number of samples with these characteristics in order to reach conclusions.

A previous azithromycin treatment for an STI or respiratory infection could explain the high resistance associated with azithromycin treatment [7,9,15]. Upon collecting data from the clinical story of patients with an Azi-R-MG infection, we observed that approximately half of the patients (45/89) had previously received treatment with this antibiotic.

Several studies demonstrate a high prevalence of resistant MG infection in MSM [5,7,9]. As it was previously described, we found that 52.8% of men with an Azi-R-MG were MSM. The high bacterial STI burden and frequent exposure to macrolide treatment may account for the high prevalence of macrolide-resistant MG among MSM [2].

It is important to keep on evaluating patients with a MG infection and its resistance mechanisms. The main conclusion reported in our study is that the concerning resistance to azithromycin and the increasing resistance to the second line treatment (moxifloxacin), correlated with treatment failure and the persistence of MG infection. As we previously mentioned, we did not evaluate the *gyrA* gene by sequencing for moxifloxacin resistance, unlike other studies [11, 14]. More studies with a larger number of samples to relate combinations of mutations responsible for azithromycin and/or moxifloxacin treatment failure are needed.

## FUNDING

None to declare

## CONFLICT OF INTEREST

Authors declare no conflict of interest

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