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Brief report

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Epidemiology and prevalence of mutations associated with resistance to macrolides and fluoroquinolones in *Mycoplasma genitalium* in a tertiary hospital from Madrid, Spain

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ABSTRACT

Objectives. *Mycoplasma genitalium* causes persistent sexually transmitted infections. The aims of this study were to estimate the prevalence of resistances to macrolides and fluoroquinolones in *M. genitalium* and the sexually transmitted coinfections in patients at Hospital Universitario La Paz (Madrid, Spain).

Material and methods. Patients attended between January and October 2021 were studied. Screening for sexually transmitted pathogens and detection of 23S rRNA and *parC* genes mutations were performed by real-time PCR (Allplex, Seegene™).

Results. A total of 1,518 females and 1,136 males were studied. The prevalence of *M. genitalium* was 2.1%. The macrolides resistance rate was 51.8%. The mutations found were A2059G, A2058T and A2058G. The rate of resistance to fluoroquinolones was 17.8% being the G248T mutation (S83I) the most frequent. Seven males had some sexual transmitted coinfection.

Conclusions. Although the percentage of *M. genitalium* infections is low, the high rate of resistance to macrolides makes it necessary to revise the protocols for diagnosis and empirical treatment of sexually transmitted infections. The use of fluoroquinolones is appropriate after screening of macrolide resistance profile.

Keywords: *Mycoplasma genitalium*, macrolides, coinfection, azithromycin, fluoroquinolones, moxifloxacin, resistance, mutation, STI

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Epidemiología y prevalencia de mutaciones asociadas a la resistencia a macrólidos y fluoroquinolonas en *Mycoplasma genitalium* en un hospital terciario de Madrid, España

RESUMEN

Objetivos. *Mycoplasma genitalium* causa infecciones de transmisión sexual persistentes. Los objetivos de este trabajo fueron estimar la prevalencia de resistencias a macrólidos y fluoroquinolonas en *M. genitalium* así como las coinfecciones de transmisión sexual en pacientes del Hospital Universitario La Paz (Madrid, España).

Material y métodos. Se estudiaron pacientes atendidos entre enero y octubre de 2021. El cribado de patógenos de transmisión sexual y la detección de mutaciones de los genes ARNr 23S y *parC* se realizaron por PCR en tiempo real (Allplex, Seegene™).

Resultados. Se estudiaron 1.518 mujeres y 1.136 hombres. La prevalencia de *M. genitalium* fue del 2,1%. La tasa de resistencia a macrólidos fue del 51.8%. Las mutaciones encontradas fueron A2059G, A2058T y A2058G. La tasa de resistencias a fluoroquinolonas fue del 17.8% siendo la mutación G248T (S83I) la más frecuente. Siete hombres presentaron alguna coinfección de transmisión sexual.

Conclusiones. Aunque el porcentaje de infecciones por *M. genitalium* es bajo, la elevada tasa de resistencias frente a macrólidos hace necesario modificar los protocolos de diagnóstico y tratamiento empírico de las infecciones de transmisión sexual. El uso de fluoroquinolonas es adecuado tras testar previamente el perfil de resistencia a macrólidos.

Palabras clave: *Mycoplasma genitalium*, macrólidos, coinfección, azitromicina, fluoroquinolonas, moxifloxacino, resistencia, mutación, ITS

INTRODUCTION

Mycoplasma genitalium (MG) is a sexually transmitted pathogen that mainly affects males causing persistent non-gonococcal urethritis [1]. Some studies have reported cases of proctitis or epididymitis in males with a high bacterial load [2]. The prevalence of MG infections in females is lower than males. Several reports have associated the detection of MG with pelvic inflammatory disease, cervicitis, premature rupture of membranes or miscarriage but there is no conclusive data [3]. *M. genitalium* has been detected in a high percentage of asymptomatic patients. However, according to the therapeutic guidelines for the management of sexually transmitted diseases, these patients should not be screened for MG detection [4].

In recent years, there has been an increase in MG resistances reported in different countries worldwide [5]. The prevalence of mutations associated with resistance to macrolides (MARM) or fluoroquinolones (MARF) contrasts between series depending of the local epidemiology [6-9]. A meta-analysis estimated the overall prevalence of MARM and MARF at 19% and 3.5% respectively, and dual resistance at 2% in Spain [5]. Resistance data in MG have been reported in different communities of Spain but there are no data from the Community of Madrid since 2015 [10].

The resistance of MG is due to mutations in the region V of the 23S rRNA gen level conferring resistance to macrolides or some mutations of the topoisomerase IV *parC/gyrA* genes level producing resistance to fluoroquinolones. Although the detection of MARM implies resistance to treatment, the detection of MARF does not always imply a therapeutic failure [6]. The treatment of choice for MG infections is azithromycin as first line followed by moxifloxacin as second line of treatment. However, different MG guidelines show the need to detect the presence of mutations to macrolides for anticipate the treatment failure and clinical complications in symptomatic patients [4].

The aim of the study was to estimate the prevalence of MG in patients at Hospital Universitario La Paz (HULP) between January and October 2021. For positive MG patients, the prevalence of MARM and MARF, percentage of previous treatment with macrolides or fluoroquinolones and the prevalence of sexually transmitted coinfections (STCs) were calculated.

MATERIAL AND METHODS

In a retrospective observational cohort study design, we collected the demographic, analytical and previous treatments data of patients with MG detection from the HULP database and laboratory informatics systems.

The reference method for MG resistance detection has been Sanger sequencing of genes. However, automated commercial kits are now available and allow a rapid detection of MG resistances by real time polymerase chain reaction (RT-PCR).

Genitourinary samples (first-void urines, rectal or vaginal swabs) were screened for sexual transmitted infections using RT-PCR (Allplex™ 7 STI Essential Assay, Seegene®, Seoul, Republic of Korea) including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Mycoplasma genitallium*, among others. Positive samples for MG were screened for detection of MARM and MARF. The resistances to macrolides were detected with the Allplex™ MG & AziR Assay including six mutations: A2058C, A2058G, A2058T, A2059C, A2059G and A2059T. Mutations associated with fluoroquinolones resistances were detected with Allplex™ MG & MoxiR Assay (Seegene®) including another six mutations against *parC* gene: A247C (S83R), G248A (S83N), G248T (S83I), G259A (D87N), G259C (D87H) and G259T (D87Y). The RT-PCRs were performed in combination with automated DNA extraction and PCR setup using a Microlab STARlet Liquid Handling robot (Hamilton®), according to the manufacturers' instructions and CFX96 Touch Real-Time PCR Detection System thermal cycler (BioRad®,Hercules, California).

No ethical review or approval was required for the study on human participants in accordance with institutional requirements of the retrospective studies. The data collected were obtained from clinical samples that were necessary for the clinical diagnosis of the patients.

RESULTS

During the study period 2,654 patients were screened for MG (1,518 were females and 1,136 were males) of which 56 patients (~2.1%) were positive, including 41 first-void urines (73.2%), 14 rectal swabs (25%) and one vaginal swab (1.8%).

The mean age of our patients was 31 years IQR (25-34), 44/56 patients were males (78.6%) and 12/56 patients were females (21.4%). Seven males (12.5%) of our study had some STCs with *N. gonorrhoeae* (n=4/56) or *C. trachomatis* (n=3/56) during the differential diagnosis.

The table 1 describes the mutations associated with resistance to macrolides and fluoroquinolones detected in relation with the samples analyzed, the previous treatment of patients with azithromycin or moxifloxacin, and the STCs.

Out of 56 isolates analyzed, twenty-three strains (41.1%) were classified as *wild type* strains. Twenty-nine strains out of 56 (51.8%) had mutations that conferring resistance to macrolides. The most detected MARM was A2059G in 11 out of 29 strains (36.6%) following by A2958T mutation (n=10/29, 34.5%). One out of 29 strains resistance to macrolides showed two mutations in A2058G and A2059G. Seventeen (58.6%) out of the 29 patients with macrolides resistance had not been previously treated with azithromycin, ten (34.5%) of the 29 patients had received prior treatment and in two cases no record was found.

Ten out of 56 strains (17.8%) had some mutations that conferring resistance to fluoroquinolones. The most detected MARF was G248T (S83I) in 6 (60%) out of 10 strains. Five (50%) out of the 10 patients with some fluoroquinolones resistance had not been previously treated with moxifloxacin, four (40%) had received prior treatment and in one case had no record.

Table 1 **Resistance to macrolides and fluoroquinolones in *Mycoplasma genitalium* (n=56)**

PCR-resistance	Previous treatment	Patients (%)	Samples positive (n)	Patients with STCs (n)
Wild-type MG strains	N/A	23	(19) Urine, (3) Rectal, (1) Vaginal	(1) NG, (2) CT
Macrolide resistance MG strains	10	29	29	N/A
A2059G	3	10 (34.5%)	(6) Urine, (4) Rectal	(1) NG
A2058T	4	10 (34.5%)	(6) Urine, (4) Rectal	(2) NG, (1) CT
A2058G	2	8 (27.6%)	(7) Urine, (1) Rectal	N/A
A2058G + A2059G	1	1 (3.4%)	(1) Urine	N/A
Fluoroquinolones resistance MG strains*	4	10	10	N/A
G259T (D87Y)	1	2 (20%)	(2) Urine	N/A
G248T (S83I)	2	6 (60%)	(4) Urine, (2) Rectal	N/A
A247C (S83R)	N/A	1 (10%)	(1) Rectal	N/A
G259A (D87N)	1	1 (10%)	(1) Urine	N/A

STCs: sexual transmitted coinfections; NG: *Neisseria gonorrhoeae*; CT: *Chlamydia trachomatis*; N/A: not applied
***Amino acid position changes are reported according to the amino acid positions within the MG G37 genome**

Dual mutations conferring resistance to macrolides and fluoroquinolones were detected also in ten strains (17.8%): three A2059G/G248T (30%), three A2058G/G259T (30%), two A2058G/G259T (20%), one A2059G/A247C (10%) and one A2058T/G259A (10%).

DISCUSSION

According to other studies, the highest prevalence of MG in HULP was detected in males of sexually active age. In our study, we found a higher prevalence of resistances to macrolides and fluoroquinolones than in other areas of Spain [6-9]. However, we have not found any predominant resistance mutations in patients with any STCs.

Studies reported in other areas of Spain showed a prevalence of macrolides resistance in MG of around 25-30% [6-9]. These data may be biased between studies by the overall number of patients screened for MG, the number of MG strains tested, the clinical patient condition or the use of macrolides in each health area. Our high MARM detected could be explained by the use of empirical treatment with azithromycin (1g single dose) in our area until 2022 for urethritis.

In our studio, a higher percentage of mutation A2058T macrolide resistance-associated was detected than in other series which the A2058G or A2059G mutations are the most frequent detected [6-8]. A higher prevalence of the A2058T mutation was also reported by Asenjo et al. in Spain or Braam et al. in Netherlands [9,10]. In future studies, it would be necessary to investigate the phylogenetic relationship between A2058T isolates.

In relation to fluoroquinolones resistance, there are few data available due to the short time of commercial kits have been on the market. In some studies from Spain, the prevalence of MARF is reported around 10% [6,7,8]. Our data shows a higher prevalence, however the number of strains tested was low (n=10) to infer globally in our population. However, mutations in the *gyrA* gene were not detected with this commercial kit, so there may be underdiagnosis in the detection of MARF. Furthermore, the detection of mutations relationship with fluoroquinolones resistance is not always associated with therapeutic failure [6].

Analysis of dual resistance to macrolides and fluoroquinolones did not give any group of mutations more prevalent than the others as suggested by other studies [6].

Most of the patients in our study with any macrolides or fluoroquinolones resistance had not received prior treatments. However, it should be noted that non-electronic prescriptions, treatments in clinics specialist in sexually transmitted diseases or single-dose treatments with azithromycin in emergency departments were not recorded in our informatic laboratory system. Therefore, there is probably an underestimation of patients previously treated with macrolides or fluoroquinolones in our area.

This study has some limitations, such as the lack of clinical data or information on patients' sexual practices, and analysis of post-treatment control. However, the reported data support that empirical use of azithromycin is not adequate for the treatment of MG infections in our population. As determined by the STI guidelines, prior to the detection of macrolides resistance, the empirical treatment should be with doxycycline (100mg/12h) to reduce the MG load and then, after the macrolide's resistance profile is known, switched the

treatment to azithromycin or moxifloxacin [11].

Future studies are needed to evaluate the impact of these measures in the prevalence of resistances to macrolides and fluoroquinolones.

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None to declare

CONFLICT OF INTEREST

The authors declare no have conflict of interest

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