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## INTRODUCTION

*Mycoplasma genitalium* is a sexually transmissible infection (STI) that has been implicated in non-gonococcal urethritis in men, and cervicitis and pelvic inflammatory disease in women. Furthermore antibiotic resistance, specially to macrolides, is alarmingly emerging in *M. genitalium* (MG). A previous study among a 2013-2014 cohort of patients in Barcelona reported an overall macrolide resistance rate of 35%, particularly marked in men who had sex with men (MSM)<sup>1</sup>. Apart from that, no large data has been published regarding to the current prevalence of these resistances in *M. genitalium* neither in Spain nor in the rest of the Mediterranean Europe.

Therefore, the aim of this study was to estimate the prevalence of macrolide resistance mediating mutations in *M. genitalium* in Barcelona, Spain, using a novel multiplex qPCR assay (ResistancePlus™ MG)<sup>2</sup>; and to evaluate macrolide resistance tendency in our area.

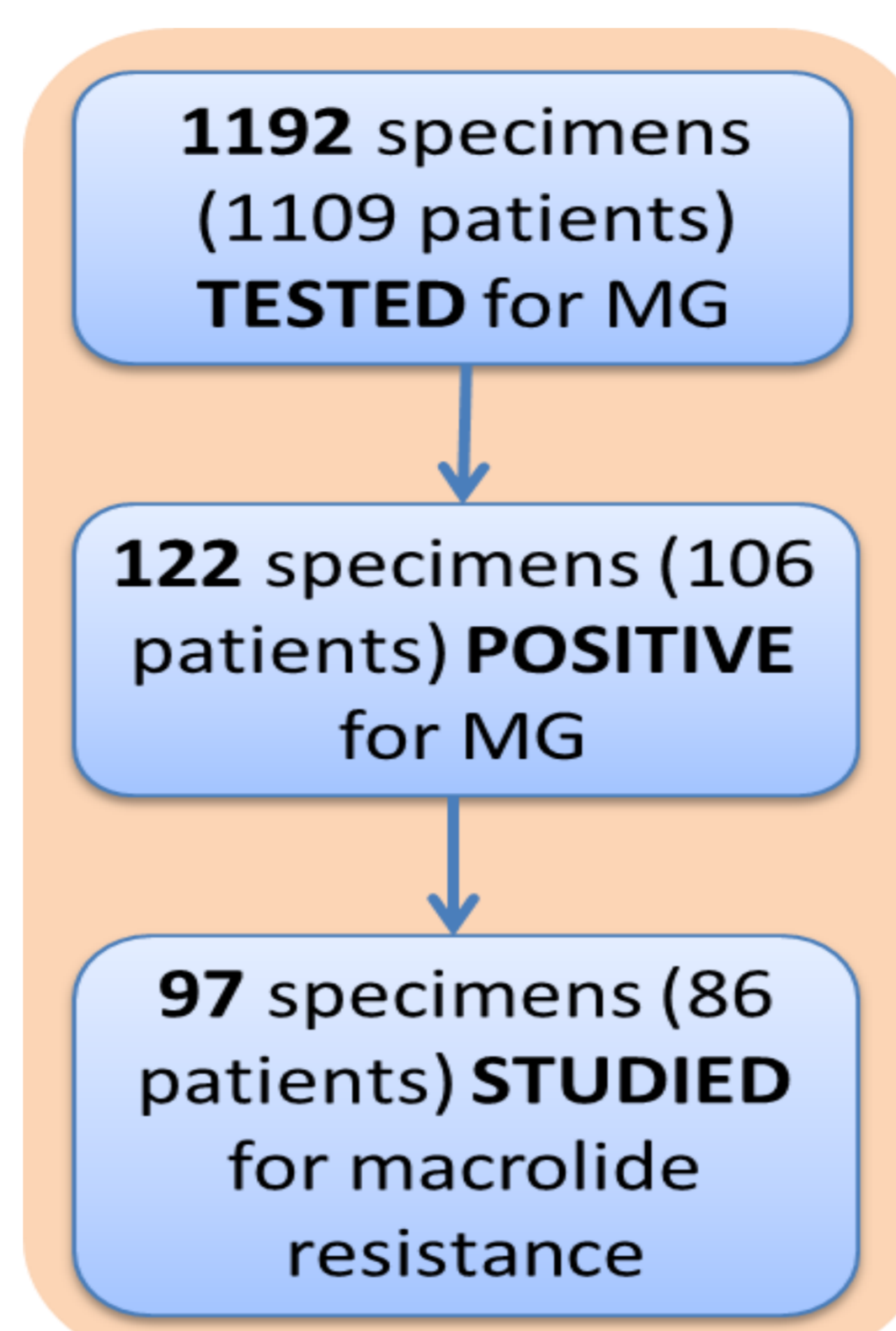
## METHODS

**Study period:** this study was conducted retrospectively with specimens submitted between December 2016 and February 2017 to the Microbiology Department of the Vall d'Hebron University Hospital.

**Molecular methods for detection of *M. genitalium* and macrolide resistance mediated mutations:** *M. genitalium* was detected using nucleic acid amplification methods (ANYPLEX II STI-7 V1-1 kit, Seegene). Genotypic macrolide resistance markers were detected through a new multiplex qPCR assay (ResistancePlus™ MG) that detects both *M. genitalium* and resistances. Finally, DNA sequencing of the 23S ribosomal RNA gene (GATC Biotech AG) was performed to confirm macrolide resistance mutations in some of the resistant *M. genitalium* positive specimens.

**Patients characterization:** demographic, baseline and clinical characteristics of the patients were collected through a comprehensive review of the medical record.

## RESULTS



**I. Samples (Figure 1):** A total of 1,192 samples were tested by ANYPLEX. Among them, 97 MG positive samples were included in the study. Table 1 shows patients data stratified by sex and sample.

Figure 1. Study Samples workflow

Only in four (4.1%) of the 97 specimens studied we obtained discordances between a positive result for *M. genitalium* by ANYPLEX kit versus a negative result through the ResistancePlus™ MG assay, likely due to low bacterial load.

Table 1. Characteristics of the patients and episodes caused by *M. genitalium*.

Characteristic	Episodes in Men	Episodes in Women
	(n = 64)	(n = 22)
	No.; % (95% CI)	No.; % (95% CI)
Median Age (range)	35 (20-69)	28 (19-41)
Infection site		
- US/FVU/S	43; 66 (5-78)	0
- V/ES	0	22; 100 (85-100)
- Rectum	17; 27 (16-39)	0
- PS	1; 2 (0-8)	0
- US/FVU/S & Rectum	3; 5 (1-13)	0

CI, confidence interval; US/FVU/S, urethral swab/first-void urine/semen; V/ES, vaginal/endocervical swab; PS, pharyngeal swab.

**II. *M. genitalium* macrolide resistance:** Macrolide resistance mediated mutations were detected in 30 (36.1%; 95% CI, 26%-46%) *M. genitalium* cases by the multiplex qPCR assay.

The 23S ribosomal RNA gene sequencing confirmed 20 of the *M. genitalium* resistant cases (Figure 2).

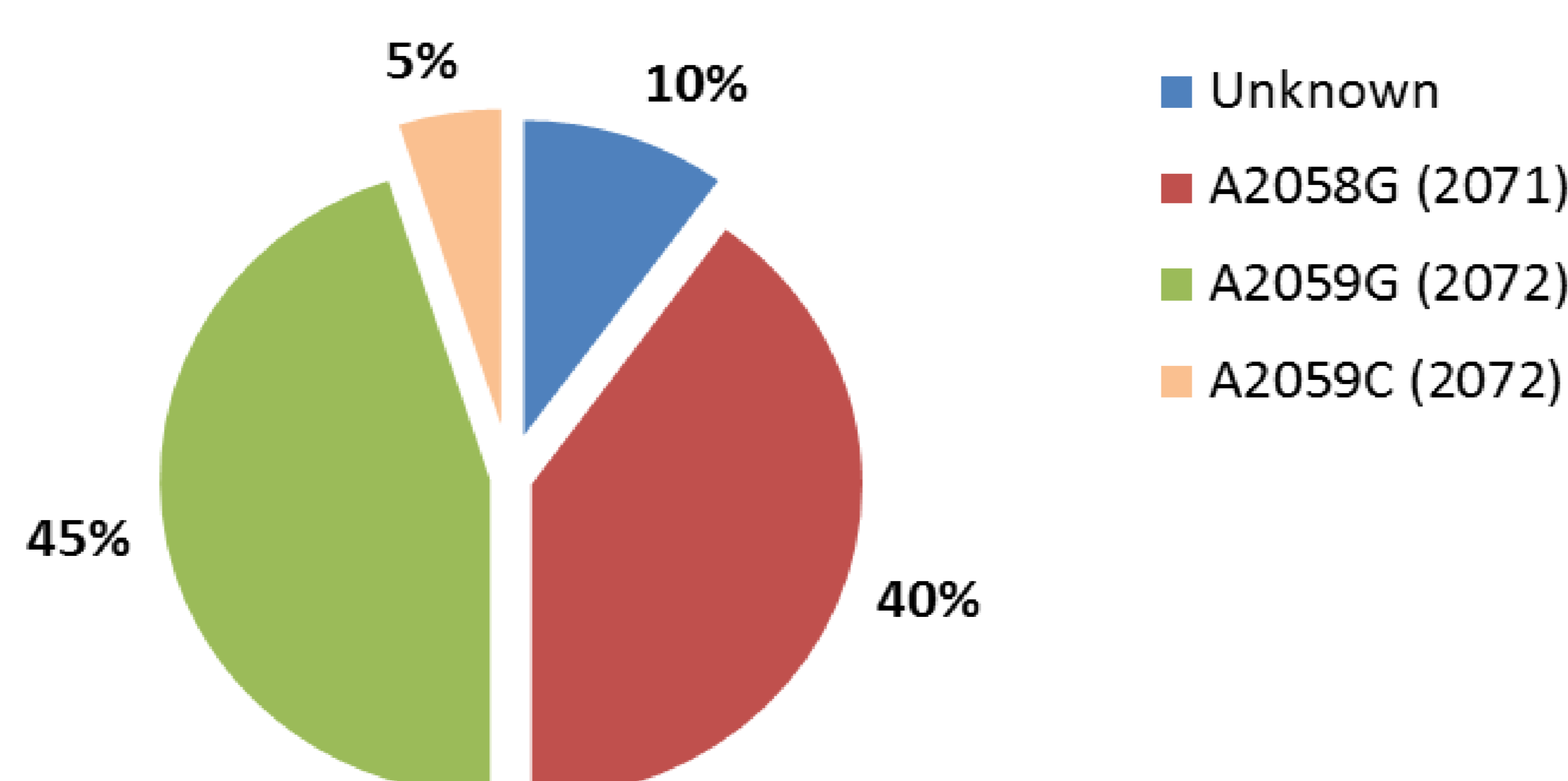


Figure 2. Macrolide resistant associated mutations in 23S ribosomal RNA gene sequencing using *E. coli* numbering. *M. genitalium* numbering is shown in parentheses.

**III. Tendency of macrolide resistance in our area:** Prevalence results of the 2016-17 cohort studied were compared, using T-student statistical test, with those from the 2013-2014 cohort<sup>1</sup> to try to elucidate whether macrolide resistance is increasing in our area (Table 2).

Table 2. Univariate analyses of the prevalence of macrolide resistance between the 2013-14 and the 2016-17 cohorts.

Prevalence of Macrolide resistance-associated mutations	% (95% CI)	Univariate Analyses P
<b>TOTAL</b>		0.9
- 2013-14 cohort	35 (24-46)	
- 2016-17 cohort	36 (26-46)	
<b>MEN</b>		0.68
- 2013-14 cohort	41 (29-54)	
- 2016-17 cohort	45 (33-58)	
<b>WOMEN</b>		0.77
- 2013-14 cohort	13 (4-29)	
- 2016-17 cohort	10 (3-22)	

Results stratified by gender point that resistances may be stable with high levels of macrolide resistance among men (specially MSM) while these resistances would be much lower among women and has not increased between both cohorts.

## CONCLUSION

The widespread appearance of macrolide resistance, also in Spain, makes imperative the implementation of combined diagnostic-resistance detection assays for *M. genitalium* in order to facilitate the optimization of antibiotic treatment in the management of non-gonococcal urethritis and potentially reduce the transmission of macrolide resistance.

## BIBLIOGRAPHY

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2. Tabrizi SN, Su J, Bradshaw CS, *et al.* Prospective-evaluation of RresistancePlus™ MG, a new multiplex qPCR assay for the detection of *Mycoplasma genitalium* and macrolide resistance. J Clin Microbiol. 2017.

