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BRIEF REPORT

Multi-year prevalence and macrolide resistance of Mycoplasma genitalium in clinical samples from a southern Italian hospital

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Abstract

The use of azithromycin for the treatment of *Mycoplasma genitalium* infections has led to resistance to macrolides. From July 2014 to July 2020, 7150 samples were analysed for the detection of sexually transmitted infections at the Policlinico of Bari. A total of 67/7150 samples (0.93%) were positive for MG DNA and 47 samples were analysed for the evaluation of six azithromycin resistance-associated mutations. In 5/47 samples, the A2058G mutation was detected (10.63%). Although the cases of positive MG samples and mutations are low in our reality, diagnostic tests to detect azithromycin resistant-associated genes may provide a convenient way to monitor resistance rate.

Keywords Mycoplasma genitalium · Epidemiology · Surveillance · Azithromycin resistance · Multiplex real-time PCR

Mycoplasma genitalium (MG) is a sexually transmitted microorganism causing clinical diseases mostly in men. Its role in nongonococcal urethritis (NGU) in men is well established and it has also been associated with pelvic inflammatory diseases, cervicitis, and preterm birth in women. MG has no peptidoglycan-containing cell wall, and thus it is naturally resistant to antibiotics such as beta-lactams; its treatment options are limited to tetracyclines, macrolides, streptogramins, or fluoroquinolones. However, due to their high therapeutic failure rate, tetracyclines are not an adequate treatment for MG infections [1].

Azithromycin regimen is usually used as first-line treatment of MG infections. Unfortunately, failure of azithromycin treatment has been reported in many cases of MG-positive NGU [2, 3]. Treatment failure may be caused by the selection of resistant mutants during treatment with a single 1-g dose of azithromycin and/or the presence of pre-existing resistant isolates [4, 5]. Several resistance-associated mutations are known to alter the ribosome binding of macrolides to the residues of 23S rRNA (*Escherichia coli* numbering): A2058G, A2058T, A2058C, A2059G, and A2059C.

The aim of the following work was to retrospectively evaluate the presence of azithromycin resistance-associated mutations in MG-positive clinical samples.

From June 2014 to the end of July 2020, 7150 specimens collected from 5624 patients (4768 females and 856 males) were processed in the U.O.C. Microbiology and Virology, Azienda Ospedaliero-Universitaria, Policlinico of Bari for the detection of sexually transmitted infections (STI). Specimens included cervical swabs, seminal fluids, male urethral swabs, endometrial fluid, anal swabs, glan swabs, and vaginal swabs (Table 1).

DNA was extracted with automated technique using MagNa Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics GmbH, Mannheim, Germany), performed on the MagNa Pure Compact System (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions, and processed by a multiplex real-time PCR assay, Anyplex STI-7™ (Seegene, Inc., Seoul, Korea), by Bio-Rad real-time CFX96TM PCR cycler (Bio-Rad Laboratories, CA, USA) [6].

Subsequently the positive samples for MG were tested using a real-time multiplex PCR assay Allplex™ MG & AziR assay (Seegene, Inc., Seoul, Korea) for the evaluation of resistance to azithromycin. This kit allows detection of six azithromycin resistance-associated mutations: A2058G,

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Table 1 Yearly prevalence of *M. genitalium* infection detected from cervical swabs, seminal fluids, male urethral swabs, endometrial fluid, anal swabs, glan swabs, and vaginal swabs. The percentage of macrolides-resistant *M. genitalium* strains was evaluated on the yearly total of *M. genitalium* strains analyzed. Only mutation A2058G was detected

Year	Analysed samples (*N*)	M. genitalium *N* samples (%)	Macrolides-resistant *M. genitalium* *N* (%)
2014*	590	1 (0.17%)	0/1 (0.00%)
2015	1223	5 (0.41%)	0/2 (0.00%)
2016	1164	15 (1.29%)	1/10 (10.00%)
2017	1271	14 (1.10 %)	1/10 (10.00%)
2018	1311	11 (0.84%)	1/8 (12.50%)
2019	1150	17 (1.48%)	1/13 (7.69%)
2020*	441	4 (0.91%)	1/3 (33.33%)
Total	7150	67 (0.94%)	5/47 (10.64%)

*Data collection started from the half of June 2014 until the end of July 2020

A2058T, A2058C, A2059G, A2059T, A2059C. An endogenous human gene is used as internal control (IC) to monitor the whole process of sample collection and nucleic acid extraction and to check for any possible PCR inhibition. The kit is validated for urine, genital swab, and liquid-based cytology specimens. The PCR runs were always accompanied with PC (positive control) and NC (negative control). Assay run is determined as valid when the fluorophores of the positive control are ≤ 45 Ct value.

The study number 5930 was approved by Ethical Committee of Policlinico of Bari (No. 0038512|06|05|2019).

In total, 67/7150 samples (0.93%), collected from 56 patients (41 females and 26 males), were MG DNA positive. Among the 56, two types of different samples were sometimes collected on the same day. The overall prevalence of MG-positive DNA was 1.00% (56/5624) and it was 0.85% in females and 3.03% in males (Fisher's exact test p value < 0.001, Cramer's $V = 0.072$). The median age of MG-positive patients was 26.50 (range 19–50). The median age of males and females was 31.0 (range 20–39) and 26.0 (range 19–50), respectively. The age difference between males and females was not statistically significant (Mann-Whitney test p value = 0.347).

The detection of macrolides resistance-associated mutations was performed on 47 samples collected from 47 patients (15 males and 32 females, 31.91% and 68.09%, respectively), excluding repeated samples and those whose DNA extract was exhausted. In particular, the following samples were analysed: 6 seminal fluids, 8 male urethral swabs, 24 cervical swabs, 1 glan swab and 3 endometrial fluids, 5 vaginal swabs.

In 5/47 samples collected from three females and two males, the A2058G mutation was detected (10.63%, 95% CI: 3.98–23.89%, 3 cervical swabs, 1 male urethral swab, and 1 seminal fluid). Two of the female patients with A2058G mutation were also positive for UU-UP and CT-UP DNA, respectively. One sample cervical swab was collected from a hospitalized patient while the others were collected from outpatients.

The meta-analysis of Machalek et al. revealed that the global prevalence of mutations associated with azithromycin resistance in MG increased from 10% before 2010 to 51% in 2016–2017. The prevalence is exceptionally high in Australia (66%) and Japan (69%) compared with data in most European countries [7]. This increased trend is probably caused by widespread use of single-dose azithromycin since the 1990s for both syndromic and aetiological management of STIs.

The most common mutations that confer macrolide resistance in MG are A2071G (*Escherichia coli* numbering 2058) and A2072G (*E. coli* numbering 2059) in the 23S ribosomal RNA (rRNA) gene, known as macrolide resistance mediating mutations (MRMMs). High-level resistance to macrolides is associated with point mutations in domain V of the 23S rRNA gene at positions 2058 and 2059 in several bacterial species [8]. In a study of Arabella Touati et al., resistant genotypes were detected in 19 patients. In particular, the mutations A2059G and A2058G were detected in 9 and 6 patients, respectively [9]. Also in this work, the A2058G mutation was found among the samples analysed at the Azienda Ospedaliero-Universitaria Policlinico of Bari.

A major limitation of this work was the absence of patients' clinical data making impossible to check the presence of symptoms related to the infection and the outcome of the treatment.

Although the prevalence of MG infection is low, it is comparable to the results of other studies [3, 10, 11]. Despite the samples bearing the mutations in the 23s rRNA gene region are few, this is the first investigation demonstrating the presence of the A2058G mutation in Apulia. Since MG has high propensity to develop antimicrobial resistance, a program of infection control is strictly required. The recent spread of azithromycin resistance in MG focuses the need to move away from the use of single-dose azithromycin to treat STIs and to consider combination therapy to improve antimicrobial efficacy. In the meantime, it is essential to monitor the spread of mutations that confer azithromycin resistance in MG in order

to quickly detect an eventual increase of the resistance rates to avoid ineffective antibiotic treatments.

Compliance with ethical standards

The study number 5930 was approved by Ethical Committee of Policlinico of Bari (No. 0038512|06|05|2019).

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