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Fluoroquinolone and Macrolide resistance-associated mutations in *Mycoplasma genitalium*

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Background: We analyzed the macrolide and fluoroquinolone resistance in *M. genitalium* positive samples collected from symptomatic patients, attending an STI center in Milan (Italy) between March 2017 and October 2019.

Materials/methods: A Total of 99 *M. genitalium* positive samples (73% males and 26% females), including 71 urethral swabs, 25 vaginal swabs and 3 anal swabs, were analyzed at the Virology Laboratory of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. The samples were tested using two commercially-available multiplex qPCR assay (ResistancePlus™ MG, Speedx and Allplex™ MG & AziR assay, Seegene) for simultaneous detection of *M. genitalium* and mutations responsible for resistance to macrolides in region V of the 23S rRNA. Moreover, 99 samples were analyzed with Allplex™ MG & MoxiR assay, Seegene to evaluate clinical performance of new assay. The resistant specimens were subsequently sequenced with the Sanger method.

Results: From 99 *M. genitalium* positive specimens 52 (52.5%) and 51 (51.5%) resulted resistant to macrolide with the ResistancePlus™ MG kit and MG & AziR assay, Seegene respectively. The presence of a single point mutation was confirmed in 51 of 52 samples with the Sanger Sequencing, while 1 sample resulted wild type (WT). The most common mutations were A2059G (35/51, 68.6%) and A2058G (10/51, 19.6%), while the least frequent were A2058T (7/51, 13.7%).

Moreover, 14/99 (14.1%) *M. genitalium* positive specimens resulted positive for moxifloxacin resistance with a frequency of mutations of G259A (4/14, 28.6%), G248T (4/14, 28.6%), G259T (3/14, 21.4%), G248A (2/14, 14.3%), A247C (1/14, 7.1%).

Interestingly, 11/99 (11,1%) positive specimen presented both macrolide and fluorochinoloni resistance.

Conclusions: *M.genitalium* was found to represent an important microbial pathogen in patients presenting with genital syndRomes in Milan, Italy. In Italy azithromycin is still widely used for the treatment of *Chlamydia trachomatis* infections, therefore a rise of the percentage presented in this analysis is expected in the next few years. Our findings support the need for ongoing antibiotic resistance surveillance and the importance of using molecular assays to tailor treatment.

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