Background: *Ureaplasma urealyticum* and *Mycoplasma hominis* belong to a group of microorganisms that are commonly found in the genitourinary tract of sexually active women as part of their normal genital tract flora. However, they have been associated with genitourinary tract infections, intrauterine and postpartum infections leading to important complications. The therapeutic efficacy of the antimicrobials used for the treatment of the infections caused by mycoplasmas is unpredictable due to increasing resistance. We aimed to evaluate the prevalence and *in vitro* susceptibility to antimicrobial agents of *U. urealyticum* and *M. hominis* isolated from vaginal samples of symptomatic reproductive age women.

Material/methods: A total of 2,988 vaginal samples from patients presenting with signs and symptoms of vulvovaginitis between 2011 and 2015 were evaluated. For the isolation and susceptibility testing of mycoplasmas, the commercial kit *Mycoplasma IST*-2 (BioMerieux, Marcy l’Etoile, France) was used according to the manufacturer’s instructions. Only concentrations of > 10^4 CFU/ml at 48h were included as positive samples in this study.

Results: Out of the 2,988 samples examined, 459 (15.4%) were positive for *U. urealyticum*, *M. hominis* or both, either alone or in combination with other pathogens. *U. urealyticum* was isolated from 385 (83.9%) of the positive samples, *M. hominis* from 19 (4.1%), while coinfection with both genital mycoplasmas was demonstrated in 55 (12.0%) subjects. The dominant clinical symptom was malodorous vaginal discharge, which was more prevalent in *M. hominis* infections compared to *U. urealyticum* (p=0.001) and coinfection of both mycoplasmas (p=0.018). Interestingly, women presenting with coinfection of *U. urealyticum* and *M. hominis* showed a higher prevalence of *Gardnerella vaginalis* compared to the group of women with *U. urealyticum* infections (p=0.005) and
M. hominis infections (p=0.008). Regarding the overall antimicrobial susceptibility of the mycoplasmas studied, doxycycline and josamycin were the most effective (98.0% and 98.5%, respectively), ciprofloxacin and ofloxacin the least effective (16.4% and 57.7%, respectively), while the susceptibility to erythromycin and azithromycin were 76.3% and 80.6%, respectively. However, M. hominis displayed higher susceptibility against ofloxacin compared to U. urealyticum (p=0.016), although the latter displayed higher intermediate resistance rate compared to M. hominis. Finally, erythromycin and azithromycin proved to be less effective against M. hominis as well as to U. urealyticum and M. hominis coinfection compared to U. urealyticum alone, where both proved to be very effective.

**Conclusions:** Genital mycoplasmas displayed decreased susceptibility rates to fluoroquinolones, erythromycin and azithromycin. Thus, these antimicrobial agents cannot be considered an option for empirical treatment. However, mycoplasmas retained an increased susceptibility rate to doxycycline and josamycin. Local surveillance of antimicrobial resistance of these species is imperative and the therapeutic approach must be guided by the *in vitro* susceptibility test results, in order to accomplish an optimal outcome.