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# Occurrence of *Mycoplasma* spp. and *Ureaplasma* spp. in genital specimens

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**ABSTRACT.** *Mycoplasma* spp. and *Ureaplasma* spp. belong to humans' genitourinary microbiota and sometimes are associated with infections of the genitourinary tract. The aim of this study was to evaluate the occurrence of *Mycoplasma* spp. and *Ureaplasma* spp. in genital specimens from patients of the 15<sup>th</sup> Regional de Saúde of Paraná State, Brazil, and to correlate the results with clinical and laboratory data. A retrospective cross-sectional study was conducted, based on the analysis of results of vaginal, endocervical, urine and urethral culture for mycoplasmas from patients attended in a reference laboratory, from January 2009 to December 2016. We evaluated 2,475 results of culture for mycoplasmas. A total of 50.8% patients were positive for mycoplasmas. Of these, 76.8% had positive culture exclusively for *Ureaplasma* spp. and 4.7% for *Mycoplasma hominis*. Both microorganisms were isolated in the microbiology culture of 18.5% of patients. Among the positive culture, 81.4% had significant concentrations. Bacterial vaginosis was the most common alteration observed in association with mycoplasmas. The high positivity of cultures for mycoplasmas, especially *Ureaplasma* spp. found in our study, highlight the presence of these microorganisms in many of the genital tract disorders that can be sexually transmitted and, consequently, should not be neglected.

**Keywords:** *Mycoplasma hominis*; *Ureaplasma* spp.; culture; infection, genitourinary tract.

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## Introduction

Mycoplasmas are small free-living microorganisms. These bacteria have no cell wall, which contributed to their classification in the mollicutes class, and have accentuated polymorphism and antimicrobial resistance to  $\beta$ -lactams (Domingues, Nogueira, Tavira, & Expósito, 2005). The lack of cell wall turns the microscopic visualization by Gram stain not applicable. However, the *in vitro* culture of mycoplasmas can be conducted in an enriched medium with precursors for biosynthesis of nucleic acids, proteins and lipids (Benfatti, Cordova, Guedes, Magina, & Cordova, 2010).

Although mycoplasmas are agents of some infections (Galvão & Souza, 2013; Bayraktar, Ozerol, Gucluer, & Celik, 2010; Benfatti et al., 2010; Domingues et al., 2005; Penna et al., 2005; Deguchi et al., 2004), they still do not have a defined role in genital infections (Montagner, Cruz, Albini, Hörner, & Bernardi, 2007). These bacteria usually affect young people in reproductive age, of low socioeconomic status, sexually active and with multiple partners (Bayraktar et al., 2010; Domingues et al., 2005). *Mycoplasma hominis* and *Ureaplasma* spp. deserve attention in lower genitourinary tract infections acquired mainly through sexual contact, such as urethritis, pelvic inflammatory disease and intrapartum infections (Benfatti et al., 2010; Deguchi et al., 2004). Infection by these microorganisms can also cause infertility in both genders, by sequelae in the reproductive system, especially in the fallopian tube and in the ability of sperm migration (Penna et al., 2005). *Mycoplasma hominis* and *Ureaplasma* spp. are often associated with bacterial vaginosis as a result of a complex interaction between aerobic and anaerobic microorganisms (Montagner et al., 2007). Studies have associated the presence of clue cells to the occurrence of mycoplasma infection, showing a relationship of these microorganisms with bacterial vaginosis (BV) (Van Belkum, Van der Schee, Van der Meijden, Verbrugh, & Sluiters, 2001; Cedillo-Ramírez, Gil, Zago, Yáñez, & Giono, 2000). In men, it is associated with nongonococcal urethritis (Deguchi et al., 2004; Domingues et al., 2005).

The colonization rate of *M. hominis* and *Ureaplasma* spp. in genital samples is quite varied, reaching 50% and 80% in sexually healthy women, respectively (Waites, Schelonka, Xiao, Grigsby, & Novy, 2009). Consequently, it is difficult to know if mycoplasmas really are the disease-causing agent (Montagner et al., 2007). In this sense, the concentration of these microorganisms in the biological sample collected must be determined to evaluate the need for treatment (Gilbert, Moellering, Eliopoulos, Chambers, & Saag, 2016). Values above  $10^3$  or  $10^4$  CCU mL<sup>-1</sup> (color change units per mL) are considered as significant bacterial concentrations, depending on the biological material (Avelar, Bertão, Pádua, Cardoso, & Siqueira, 2007; Ferreira & Avila, 2001; 2013; Fourmaux & Bebear, 1997). Erythromycin, clindamycin, doxycycline, or amoxicillin may be considered for genital mycoplasmas treatment (Gilbert et al., 2016); however, some drugs are not effective for both genera (Galvão & Souza, 2013).

Thus, this study aimed to evaluate the occurrence of *Mycoplasma* spp. and *Ureaplasma* spp. in genital specimens from patients of the 15<sup>th</sup> Regional de Saúde of Paraná State, Brazil, and to correlate the results with clinical and laboratory data.

## Material and methods

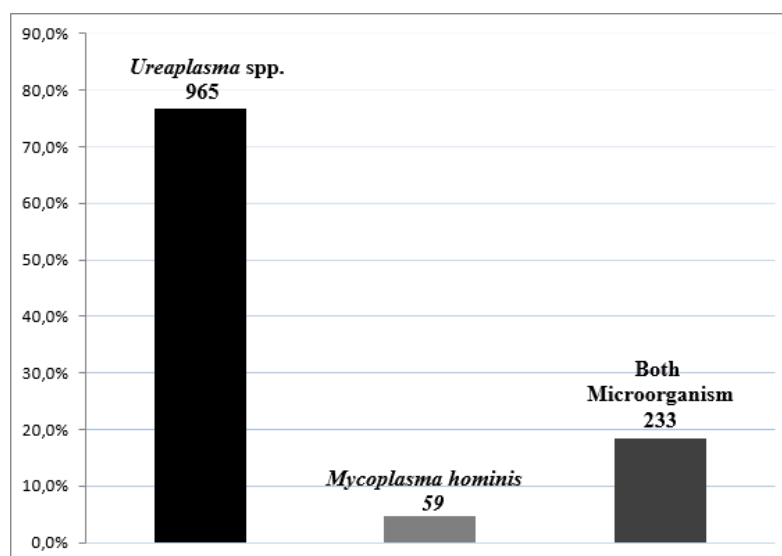
A retrospective cross-sectional study was conducted based on the analysis of results of vaginal, endocervical, urine and urethral culture for mycoplasmas from patients attended at LEPAC, a university reference laboratory, from January 2009 to December 2016.

Data of other auxiliary tests related to the diagnosis of genital tract infections were also collected. Patients' records, containing the variables gender, age, symptoms and the results of bacterioscopy test, wet mount microscopy and genital culture, were used as an instrument for data collection. The study was approved by the ethics committee of the State University of Maringá, Paraná, Brazil (protocol 353,099). Thus, in this study, values  $\geq 10^3$  CCU mL<sup>-1</sup> for vaginal secretions and urine, and  $\geq 10^4$  CCU mL<sup>-1</sup> for endocervical specimens (color change units per mL) of mycoplasmas were considered significative values (Avelar et al., 2007; Ferreira & Avila, 2001; 2013; Fourmaux & Bebear, 1997).

Using Microsoft Excel™ *software*, a spreadsheet was made with the collected variables, and data analysis was carried out using descriptive statistics.

## Results

A total of 2,475 test results were analyzed, from which 2,421 (97.8%) were female and 54 (2.2%) male patients. *Mycoplasma hominis* and *Ureaplasma* spp. were detected in 1,257 (50.8%) patients. Of these, 965 (76.8%) and 59 (4.7%) had positive cultures exclusively for *Ureaplasma* spp. and *M. hominis*, respectively. Both *M. hominis* and *Ureaplasma* spp. were detected in 233 (18.5%) patients (Figure 1).



**Figure 1.** Occurrence of *Mycoplasma hominis* and *Ureaplasma* spp. in genital samples from patients attended at Laboratório de Ensino e Pesquisa em Análises Clínicas at State University of Maringá (LEPAC/UEM), Brazil, from January 2009 to December 2016.

The highest rate of mycoplasmas occurred in patients aged from 25 to 37 years (39.3%). However, cases were observed in patients who were from 12 to 76 years old. The lowest rate was in patients older than 62 years old.

From the 1,257 mycoplasmas positive cultures analyzed, 1,024 (81.4%) had significant concentrations when considering values above  $10^5$  or  $10^4$  CCU mL<sup>-1</sup> for *M. hominis* and/or *Ureaplasma* spp. Among these, 27 patients were pregnant women and 59.3% had clinically significant concentrations for mycoplasmas.

The most common patients' complaint was vaginal or urethral discharge, reported by 1,871 (75.6%) patients. Of these, 987 (52.8%) had mycoplasma detected by culture, but significant concentrations were detected in 847 (45.3%) patients.

Gram stain from a vaginal specimen was performed in 577 (45.9%) patients, who had a positive culture for mycoplasma. Increased number of leukocytes (+++ to +++) was observed in 337 (58.4%) patients, and the absence or a small amount of Döderlein bacilli in 282 (48.9%).

Among the patients, *Trichomonas vaginalis* was observed in thirteen exams, seven (53.8%) of which were associated with mycoplasmas. Of the 169 patients that showed yeasts and/or pseudohyphae by Gram stain, 76 (45.0%) were associated with mycoplasma. Bacterial vaginosis was the alteration mostly found in association with mycoplasmas (76.3%).

Alongside the culture for mycoplasma, cultures for other common etiologic agents were performed in 1,119 (45.2%) patients. In 19 (1.7%), other cultures were detected: *Enterococcus faecalis*, *Candida albicans*, *Candida tropicalis*, *Streptococcus agalactiae*, *Klebsiella pneumoniae* and *Neisseria gonorrhoeae*. Of these, eight (42.1%) patients, all female, had some of the above microorganisms associated with mycoplasmas.

## Discussion

Mycoplasma genital presence has been quite frequent and can have adverse effects on the genitourinary tract. In the agents' studies, as consequence of their presence in the genital microbiota, a constant challenge is to establish the role of these microorganisms in the pathogenesis of human disease.

The high frequency of *M. hominis* and *Ureaplasma* spp. found in this study corroborates the findings of others (Montagner et al., 2007; Domingues et al., 2005; Rodriguez et al., 2001), strengthening the common occurrence of these microorganisms in the genitourinary tract in some regions of the world. Mycoplasmas have been found in both men and women and are more frequent in women, perhaps due to the higher concern for health and the consequent demand for medical appointments (Benfatti et al., 2010). Even virgin women can be affected, presenting low concentrations of genital mycoplasmas (McCormack et al., 1986).

In our study, we observed that the most common infection pattern was *Ureaplasma* spp. mono-infection (76.8%), the second cause was coinfection pattern (18.5%) with both microorganisms, and just 4.7% had positive cultures exclusively for *M. hominis*, corroborating a study made in China in 2012 (Xiu, Zhang, Li, Wang, & Peng, 2019).

Our results showed that 59.3% of pregnant women had clinically significant concentrations for mycoplasmas. Kusanovic et al. (2019), describe that *Ureaplasma urealyticum* and *Mycoplasma hominis* are the most common bacteria found in the amniotic fluid of patients with genetic amniocentesis, short cervix, and others uterine deficiencies or deformations. This description may explain our findings and corroborate the work of other authors (Montenegro et al., 2019; Cox et al., 2016; Hassan et al., 2006).

Among the mycoplasmas, *Ureaplasma* spp. was more frequently isolated from patient's genital samples participating in our study. Differently from our results, Rodriguez et al. (2001) reported that the most frequent was *M. hominis*, particularly in women in a study performed in Spain. On the other hand, in a previous study in the same geographic region where our study was conducted, Avelar et al. (2007) found more frequency of isolation of both species simultaneously. In a study carried out in the state of Minas Gerais, Brazil, using the polymerase chain reaction (PCR) methodology, *Ureaplasma* spp. also was the most frequently detected pathogen in ectocervical and endocervical samples (Rodrigues et al., 2011).

*Ureaplasma* spp. and *M. hominis* have been implicated in a variety of clinical conditions primarily related to lower genital tract colonization and infection. A study made in India reported that 28.2% of the patients with urogenital infections were infected by genital mycoplasmas (Rawre et al. 2019).

The simple detection of mycoplasma in a genital sample does not differentiate colonization from disease. Most colonized individuals remain asymptomatic, but there are considerable evidences that *M. hominis* and *Ureaplasma* spp. can also cause diseases (Rawre et al., 2019; Schlicht et al., 2004). Thus, mycoplasma quantitative culture is relevant, and it can be carried out by a microtiter method based on CCU

mL<sup>-1</sup> in the sample. Ferreira and Avila (2013) considered cut-off concentrations values, for both microorganisms,  $\geq 10^3$  CCU mL<sup>-1</sup>, for vaginal secretions and urine and  $\geq 10^4$  CCU mL<sup>-1</sup> for endocervical specimens (Rawre et al. 2019; Avelar et al., 2007; Ferreira & Avila, 2001; 2013; Fourmaux & Bebear, 1997). Other authors consider significative values concentrations  $\geq 10^5$  CCU mL<sup>-1</sup> (Oplustil, Zoccoli, Tobouti, & Sinto, 2010; Raddi, Rossi, & Moreira, 1989; McCormack et al., 1986). Once there is no agreement among significative values between authors, other laboratory findings and patients' clinical data are essential for the correct valuation of these agents.

Analyzing the cut-off points above mentioned, in the present study, 42.4% of the attended patients had significative values of mycoplasmas. For colonized patients, treatment is not indicated. Therefore, establishing whether the patient is infected or colonized is very important for the correct clinical management.

In this study, from the patients who complained of increased genital secretion, 45.3% had mycoplasma concentrations  $\geq 10^3$  CCU mL<sup>-1</sup>. These data corroborate the research conducted by Bayraktar et al. (2010). However, other studies have also detected asymptomatic women with mycoplasma concentrations  $\geq 10^3$  CCU mL<sup>-1</sup> (Galvão & Souza, 2013; Bayraktar et al., 2010; Benfatti et al., 2010; Domingues et al., 2005; Penna et al., 2005; Deguchi et al., 2004). Also, we observed marked leucocyte presence in most patients who had cultures with concentrations  $\geq 10^3$  CCU mL<sup>-1</sup> for *M. hominis* and *Ureaplasma* spp. This can be an auxiliary factor for the diagnosis of genital infections by these microorganisms, as previously reported in another study (Montagner et al., 2007).

Bacterial vaginosis was the most frequent disturb found in association with mycoplasmas. The presence of other microorganisms such as *E. faecalis*, *C. albicans*, *S. agalactiae*, *C. tropicalis* and *K. pneumoniae* have been reported in other studies (Benfatti et al., 2010; Montagner et al., 2007; Penna et al., 2005; Cordova & Cunha, 2002). Nonetheless, the studies mentioned above reported only the correlation of mycoplasmas with the presence of clue cells and yeast and pseudohyphae, which were the most commonly found microorganisms. This might indicate that clue cells produce an alkaline environment, which exfoliates epithelial cells and facilitates the adhesion of mycoplasmas (Montagner et al., 2007).

Association among *T. vaginalis* and *M. hominis* and *U. urealyticum* has been indicated as cause of genital disease (Penna et al., 2005), which corroborates the rate found in our study. In addition, the positivity of *M. hominis* and *Ureaplasma* spp. was observed in almost 60% of pregnant women. This high percentage of positive culture for mycoplasmas ( $\geq 10^3$  CCU mL<sup>-1</sup>) in these patients arise some concern. The literature shows that the chance of abortion increases fivefold when associated with bacterial vaginosis and other pathogens, such as *T. vaginalis*, *M. hominis* and *Ureaplasma* spp. (Bayraktar et al., 2010). It is evident for us that research of mycoplasmas in a vaginal sample during pregnancy is of paramount importance in order to allow the adoption of appropriate measures for treating pregnant women and preventing complications for both baby and mother (Bayraktar et al., 2010).

As the pathogenesis of *M. hominis* and *Ureaplasma* spp. have not been totally elucidated, Camilo, Monte, and Barcellos (2014) and Cedillo-Ramírez et al. (2000) suggest that trading conditions between them, which benefits either, are one of the factors that favor triggering the infection process. Also, it is possible that another microorganism that induces to disease may create favorable conditions for the growth of mycoplasmas (Camilo et al., 2014).

Since infections of different sexually transmitted pathogens often present similar clinical symptoms, it is almost impossible to distinguish them from mycoplasmas clinically. In this sense, the quantitative mycoplasma culture is essential to help elucidating this issue (Xiu et al., 2019).

## Conclusion

The high positivity of cultures for mycoplasmas, especially *Ureaplasma* spp. found in our study highlights the importance of these microorganisms in many of the genital tract disorders that can be sexually transmitted and, consequently, should not be neglected. In addition to showing the high positivity of mycoplasma cases, this study indicates the importance of continuing to study these bacteria, both in patients with genital symptoms and in asymptomatic patients.

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