ESCOLA BAHIANA DE MEDICINA E SAÚDE PÚBLICA

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# ANALYZING ANTIBIOTIC RESISTANCE PATTERNS IN *Mycoplasma hominis* and *Ureaplasma* spp.: INSIGHTS FROM A PRIVATE LABORATORY IN SALVADOR, BAHIA, BRAZIL

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Trabalho de Conclusão de Curso apresentado à Escola Bahiana de Medicina e Saúde Pública, como parte dos requisitos para obtenção do título de Bacharel em Biomedicina.

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Este Trabalho de Conclusão de Curso foi julgado adequado à obtenção do grau de Bacharel em Biomedicina e aprovada em sua forma final pelo Curso de Biomedicina da Escola Bahiana de Medicina e Saúde Pública.

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*“Meu bem querer*

*É segredo, é sagrado*

*Está sacramentado em meu coração”*

- Djavan

# RESUMO

*Mycoplasma* e *Ureaplasma* são patógenos associados a infecções do trato urogenital em homens e mulheres sexualmente ativos, mas são pouco estudados em comparação a outras infecções sexualmente transmissíveis, resultando em perfis de sensibilidade e resistência antimicrobiana mal definidos.

Este estudo teve como objetivo avaliar o perfil de sensibilidade do *Mycoplasma hominis* e *Ureaplasma* spp. em pacientes de um laboratório privado em Salvador, Bahia, entre março de 2022 e março de 2024, através de dois testes diagnósticos: o IST2 e o IST3. Foram identificados 819 indivíduos positivos para a cultura de micoplasmas, dos quais 262 foram selecionados para análise descritiva de dados, incluindo informações sobre sexo, idade, ano do diagnóstico e perfil de sensibilidade. Os dados foram armazenados em um banco de dados eletrônico através do sistema REDCap (Research Electronic Data Capture).

Os resultados revelaram que *Mycoplasma hominis* e *Ureaplasma spp*. atingem predominantemente mulheres (98,47%) e indivíduos com idades entre 38 e 47 anos, sendo *Ureaplasma* responsável pela maior frequência de casos positivos. Observou-se que 75% de *Mycoplasma hominis* e 84,16% de *Ureaplasma urealyticum* eram resistentes à ciprofloxacina no teste IST2, com coinfecções aumentado a resistência para 95%. No teste IST3, *Ureaplasma* spp. apresentou resistência de 7,29% à levofloxacina, com coinfecções aumentando a resistência para 22,22%. Esses achados ressaltam a necessidade urgente de intervenções de saúde pública e estratégias diagnósticas adaptadas para lidar com o aumento da resistência a antibióticos em populações vulneráveis.



Palavras-chave: *Mycoplasma hominis*; *Ureaplasma* spp; Epidemiologia; Sensibilidade.

**SUMÁRIO**

1. ARTIGO CIENTÍFICO

# ANALYZING ANTIBIOTIC RESISTANCE PATTERNS IN *Mycoplasma hominis* and *Ureaplasma* spp.: INSIGHTS FROM A PRIVATE LABORATORY IN SALVADOR, BAHIA, BRAZIL

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Abstract

Purpose

*Mycoplasma* and *Ureaplasma* are pathogens associated with urogenital infections in sexually active men and women, but they are less studied compared to other sexually transmitted infections, resulting in poorly defined antimicrobial susceptibility and resistance profiles.

Methods

This study aimed to evaluate the susceptibility profile of *Mycoplasma hominis* and *Ureaplasma* spp. in patients from a private laboratory in Salvador, Bahia, Brazil, between March 2022 and March 2024, using two diagnostic tests: IST2 and IST3. A total of 819 individuals tested positive for mycoplasma culture, of which 262 were selected for descriptive data analysis, including information on sex, age, year of diagnosis, and susceptibility profile. Data were stored in an electronic database using the REDCap (Research Electronic Data Capture) system.

Results

The results revealed that *Mycoplasma hominis* and *Ureaplasma* spp. predominantly colonize women (98.47%), and individuals aged between 38 and 47 years, with *Ureaplasma* accounting for the highest frequency of positive cases. It was observed that 75% of *Mycoplasma hominis* and 84.16% of *Ureaplasma urealyticum* were resistant to ciprofloxacin in the IST2 test. In the IST3 test, *Ureaplasma spp*. showed a resistance rate of 7.29% to levofloxacin, with co-infections increasing resistance to 22.22%.

Conclusion

These findings underscore the urgent need for public health interventions and adapted diagnostic strategies to address the rising antibiotic resistance of these species in the vulnerable populations.

**Keywords**: *Mycoplasma hominis*; *Ureaplasma* spp; Epidemiology; Resistance.

## 1.1 Introduction

*Mycoplasma* and *Ureaplasma*, commonly referred to as "Mycoplasmas," are prokaryotes belonging to the phylum Tenericutes, class Mollicutes. They are unique due to their lack of a cell wall, which makes them resistant to traditional Gram staining methods and contributes to their pleomorphic nature and have a low metabolism and rapid multiplication [1, 2]. They have a plasma membrane composed of specific lipids known as sterols, which enhance the membrane's stability and make it more resistant to osmotic lysis compared to other bacteria [1, 3]. These prokaryotes can form multiple microfilaments and produce small colonies with a characteristic "fried egg" appearance, which are irregularly shaped, pleomorphic, light in color, and have a denser central core. [3]. Furthermore, they are characterized by difficult and slow growth in conventional culture media, as they require proteins with specific properties, which vary between species, and a high carbon concentration. [4].

The two genders, *Mycoplasma* and *Ureaplasma*, differ mainly due to Ureaplasma's ability to produce the catalytic enzyme urease, which breaks down urea into ammonia and carbon dioxide, making the environment around the cells more alkaline. [5]. The bacteria *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* are significant microorganisms within the group of Sexually Transmitted Infections (STIs), capable of affecting the urogenital tract in both sexually active men and women. [2]. In this context, when not asymptomatic, they tend to cause various infections in the genitourinary tract of sexually active men and women, such as Prostatitis, Bacterial Vaginosis, Cervicitis, Pelvic Inflammatory Disease (PID) and even infertility of both sexes [1, 4]. Furthermore, they may also be related to an increase in premature births, premature membrane ruptures, low birth weight and perinatal or neonatal death [6].

The Pan American Health Organization (PAHO) estimates that more than one million STIs, such as mycoplasmas, are acquired every day worldwide, often asymptomatically [7]. Pain during urination, discharge from the genitals, and discomfort during sexual intercourse are common symptoms associated with various STIs, including "Mycoplasmas", so there is no specific symptomatic profile for identifying it [2, 8]. Moreover, STIs caused by mycoplasmas, as they have slow growth in conventional culture media, are marked by difficulty in characterizing an epidemiological profile, between the states and regions of Brazil, for example [4].

The absence of a standardized laboratory protocol for diagnosing *Mycoplasma hominis* and *Ureaplasma* spp., specific treatment for each species remains undefined, with antibiotics recommended in therapeutic guidelines for other STIs [4]. However, due to this lack of specific treatment, there has been a persistent decline in the effectiveness of treatments for these infections, representing a global challenge [9]. This scenario highlights the urgent need for targeted studies and improved therapeutic strategies, since antimicrobial resistance has been recognized as a global health threat and a priority for public health initiatives by the World Health Organization and leading experts in the field [10, 11]

Mycoplasmas are sensitive to antibiotics that act by inhibiting protein synthesis, DNA and RNA, disrupting metabolism, and damaging cell membranes. These antibiotics fall into classes such as Tetracyclines, Macrolides, Ketolides, Lincosamides, and Fluoroquinolones [12, 13]. The scenario of treatment with a specific class of antibiotics, there is a dissemination of bacterial strains resistant to some of these drug classes, posing a clinical management challenge for these infections [14]. This has already been described in macrolide-resistant strains of *Mycoplasma genitalium* and *Ureaplasma* spp., such as azithromycin-resistant, resistance to tetracyclines and

fluoroquinolones, including levofloxacin and moxifloxacin [9]. In this context, despite the significant potential of pathogens such as *Mycoplasma hominis* and *Ureaplasma* spp. to cause infertility in both females and males, these organisms remain under-researched, particularly in Brazil, when compared to other STIs [4].

In addition, the antimicrobial susceptibility profile of these pathogens in Brazil is not well-defined or thoroughly discussed in the literature, as their resistance patterns vary geographically across different regions of the country, further complicating treatment strategies and highlighting the need for more localized studies. In this context, this study provides an opportunity to assess the antimicrobial susceptibility profile of these microorganisms in individuals who underwent testing at a private laboratory in Salvador, Bahia. It will focus in describe the epidemiological characteristics of the patients, analyzing the prevalence of individuals with detected *Mycoplasma* and *Ureaplasma* species, and detail the sensitivity profile of positive cultures.

## 1.2 Materials and methods

### 1.2.1 Study design and population

This descriptive retrospective study was conducted in Salvador, the capital of the state of Bahia (estimated population of 2,5 million in 2024), located in northeastern region of Brazil. From March 2022 to March 2024, were analyzed the database of individuals treated at a private lab in Salvador, Bahia, Brazil. During this period, a total of 8,611 Mycoplasmas tests were performed. Inclusion criteria was individuals aged 18 years or older who had a positive. In addition, individuals with incomplete or unclear records were excluded. Based on a hypothetical frequency of 50%, we estimated a minimum sample size of 262 participants in order to provide a precision of 5% with 95% confidence. Participants were randomly selected from the list of all positive Mycoplasma culture registered in the SMARTLab® system. Random selection was done using the RStudio (Posit PBC) statistical analysis system.

### 1.2.2 Data collection

All epidemiological data and diagnostic results were collected from the secondary database from the SMARTLab® system, which included information on age, sex and Mycoplasma culture results (e.g. microorganisms' identification and sensitivity profile). Data were stored electronically via REDCap (Research Electronic Data Capture)[15].

Until March 2023, were used the Mycoplasma IST2 diagnostic test (BioMérieux-France) to identify and quantify *Mycoplasma hominis* and *Ureaplasma urealyticum*, and test sensitivity to nine antibiotics, including Doxycycline, Josamycin, Ofloxacin, Erythromycin, Tetracycline, Ciprofloxacin, Azithromycin, Clarithromycin and Pristinamycin. Since March 2023, the Mycoplasma IST3 kit has been introduced, which additionally identifies Ureaplasma parvum and tests sensitivity to six antibiotics separately for each species, being: Levofloxacin, Moxifloxacin, Tetracycline and Clindamycin for *Mycoplasma hominis* and Moxifloxacin, Tetracycline, Erythromycin, Telithromycin and Levofloxacin for *Ureaplasma* spp. The study compares data from the year before and after the switch to IST3.

### 1.2.2 Statistical analysis

The data recorded was validated and organized using the REDCap (Research Electronic Data Capture) program (Vanderbilt University Medical Center, Nashville). The relative and absolute frequency were calculated by sex, age groups (18-27, 28-37, 48-57, 58-67 and 68-77), Mycoplasma culture (IST2 versus IST3 test) and antibiotic susceptibility profile. All data were analyzed using the RStudio (Posit PBC) statistical analysis system.

### 1.2.3 Ethical approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee at the Bahiana School of Medicine and Public Health (CAEE 81723824.0.0000.5544 and date of approval 09/13/2024).

## 1.3 Results

### 1.3.1 Sociodemographic data

A total of 8,611 tests were performed between the study period, of which 819 (9.51%; 819/8,611) were positive on adults for *Mycoplasma*, *Ureaplasma*, or for both microorganisms. After the randomization, 262 individuals were analyzed. The distribution by age groups were: 37 (14.12%) were 18-27 years old, 85 (32.44%) were 28-37 years old, 95(36,26%) were 38-47 years old, 32 (12,21%) were 48-57 years old, 9 (3.44%) were 58-67 years old and 4 (1,53%) were 68-77 years old. Most of the participants were female (98.47%; 258/262) (Table 1).

Among the 262 patients selected for the study, 216 tested positive for *Ureaplasma* spp. (82.4%), a smaller group of 8 (3.1%) patients were identified with *Mycoplasma hominis*. Additionally, there is a subset of 38(14.5%) patients who presented with a co-infection, testing positive for both *Ureaplasma* and *Mycoplasma*.

**Table 1**. Sociodemographic characteristics of 262 selected positive culture cases in a private laboratory in Salvador, Brazil (2022-2024).

| Characteristics | Positive test<br>*n* (%) |
|---|---|
| *Sex* | |
| Female | 258 (98.47) |
| Male | 04 (1.53) |
| *Age group* | |
| 18-27 | 37 (14.12) |
| 28-37 | 85 (32.44) |
| 38-47 | 95 (36.26) |
| 48-57 | 32 (12.21) |
| 58-67 | 09 (3.44) |
| 68-77 | 04 (1.53) |

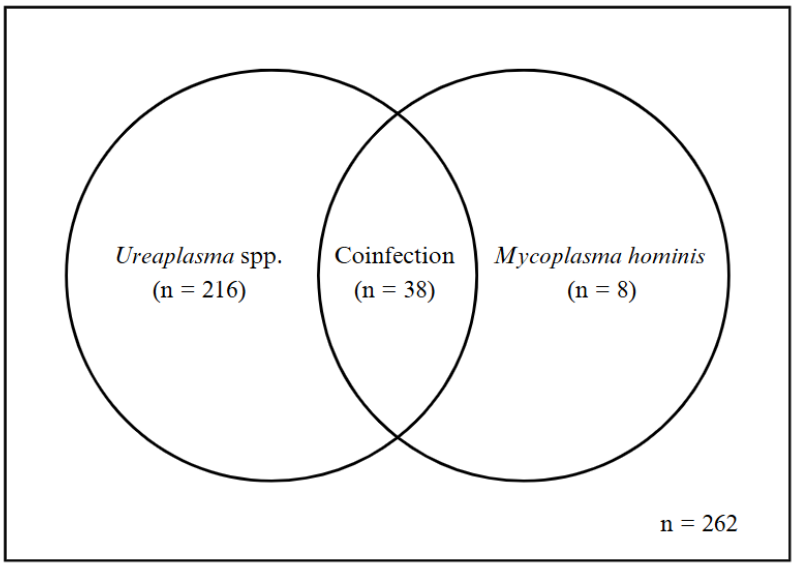


**Figure 1.** Venn diagram showing 262 randomized positive individuals categorized into *Ureaplasma* and *Mycoplasm*a

1.3.2 IST2 Test

A total of 144 individuals representing 54.96% (144/262) among the 262 individuals selected for this study were diagnosed through the Mycoplasma IST2 test. Of these individuals, 83.33% (120/144) tested positive only for *Ureaplasma urealyticum*, 2.77% (4/144) only for *Mycoplasma hominis* and 13.88% (20/144) for coinfection of both species (Table. 2).

Considering that 4 out of the 144 (2,7%) individuals tested positive only for *Mycoplasma hominis* via the IST2 test, it was observed that these individuals exhibited 75% (3/4) resistance to Ciprofloxacin and Ofloxacin indicating limited efficacy. Azithromycin showed moderate efficacy with 50% sensitivity, 25% intermediate, and 25% resistance, indicating mixed results just as Erythromycin that displayed 75% sensitivity and 25% resistance. Clarithromycin showed 75% sensitivity and 25% resistance, showing it to be kind of effective. Doxycycline, Josamycin, Tetracycline, and Pristinamycin each demonstrated 100% sensitivity against *Mycoplasm*, with no intermediate or resistant cases reported (Table. 2).

Of the 144 individuals tested using the IST2 test, 120 (83%) were positive for *Ureaplasma urealyticum*. Doxycycline, Tetracycline, and Pristinamycin each demonstrated 100% sensitivity, indicating complete effectiveness against this pathogen. Josamycin also showed high efficacy with 99.16% sensitivity and a minimal 0.83% intermediate response. Clarithromycin was effective as well, with 98.33% sensitivity and only 1.66% resistance. Erythromycin and Azithromycin exhibited high sensitivity at 95.83% and 94.16%, respectively, though Erythromycin had 2.5% intermediate and 1.66% resistant cases, while Azithromycin had 2.5% intermediate and 3.33% resistance. In contrast, Ofloxacin had only 29.16% sensitivity and a high resistance rate of 70.83%, and Ciprofloxacin showed limited efficacy with 15% sensitivity, 0.83% intermediate, and 84.16% resistance, indicating its unsuitability for treating *Ureaplasma urealyticum* infections (Table 2).

The 20 individuals out of 144 (13,88%) who tested positive for both species, *Ureaplasma urealyticum* and *Mycoplasma homins,* using the IST2 test, demonstrated a more resistance profile to the antibiotics. Ofloxacin

demonstrated low efficacy with only 10% sensitivity and a high resistance rate of 90%, while Ciprofloxacin showed similarly poor performance with 5% sensitivity and 95% resistance. Erythromycin showed 20% sensitivity, 20% intermediate, and 60% resistance, and Azithromycin displayed 20% sensitivity, 15% intermediate, and 65% resistance, suggesting limited effectiveness in mixed infections. Clarithromycin displayed only 25% sensitivity, 15% intermediate response, and 60% resistance, further indicating limited utility for treating mixed infections. Doxycycline and Josamycin each maintained 100% sensitivity, indicating high and consistent effectiveness in mixed infections. Pristinamycin also showed strong efficacy with 95% sensitivity and only a 5% intermediate response, making it effective in most mixed cases. Tetracycline followed closely, with 95% sensitivity and a minimal 5% intermediate response (Table. 2).

**Table 2:** Antibiogram for 4 positive cases of *Mycoplasma hominis*, 120 cases of *Ureaplasma urealyticum* and 20 cases of coinfection using the IST2 test, evaluating susceptibility to the following antibiotics: Doxycycline, Josamycin, Ofloxacin, Erythromycin, Tetracycline, Ciprofloxacin, Azithromycin, Clarithromycin, and Pristinamycin.

| Antibiotics | *Mycoplasm* *(n=4)* | | | *Ureaplasm* *(n=120)* | | | *Mycoplasm* and *Ureaplasm* *(n=20)* | | |
|---|---|---|---|---|---|---|---|---|---|
| | S | I | R | S | I | R | S | I | R |
| Doxycycline | 4 (100%) | 0 | 0 | 120 (100%) | 0 | 0 | 20 (100%) | 0 | 0 |
| Josamycin | 4 (100%) | 0 | 0 | 119 (99.16%) | 1 (0.83%) | 0 | 20 (100%) | 0 | 0 |
| Ofloxacin | 1 (25%) | 0 | 3 (75%) | 35 (29.16%) | 0 | 85 (70.83%) | 2 (10%) | 0 | 18 (90%) |
| Erythromycin | 3 (75%) | 0 | 1 (25%) | 115 (95.83%) | 3 (2.5%) | 2 (1.66%) | 4 (20%) | 4 (20%) | 12 (60%) |
| Tetracycline | 4 (100%) | 0 | 0 | 120 (100%) | 0 | 0 | 19 (95%) | 1 (5%) | 0 |
| Ciprofloxacin | 1 (25%) | 0 | 3 (75%) | 18 (15%) | 1 (0.83%) | 101 (84.16%) | 1 (5%) | 0 | 19 (95%) |
| Azithromycin | 2 (50%) | 1 (25%) | 1 (25%) | 113 (94.16%) | 3 (2.5%) | 4 (3.33%) | 4 (20%) | 3 (15%) | 13 (65%) |
| Clarithromycin | 3 (75%) | 0 | 1 (25%) | 118 (98.33%) | 0 | 2 (1.66%) | 5 (25%) | 33 (15%) | 12 (60%) |
| Pristinamycin | 4 (100%) | 0 | 0 | 120 (100%) | 0 | 0 | 19 (95%) | 1 (5%) | 0 |

Legend: S: sensitive; I: intermediate; R: resistant; Absence of interpretation criteria

### 1.3.3 IST3Test

Out of the 262 individuals selected for this study, a total of 118 individuals, representing 45.04% (118/262), were diagnosed using the Mycoplasma IST3 test. Among these, 81.36% (96/118) tested positive only for *Ureaplasma* spp., 3.39% (4/118) tested positive only for *Mycoplasma hominis*, and 15.25% (18/118) were diagnosed with coinfection of both species (Table 3).

For the 3.39% (4/118) individuals who tested positive solely for *Mycoplasma hominis* via the IST3 test, it was observed that none of these individuals exhibited significant resistance to the antibiotics tested. (Table 3).

Regarding 81.36% (96/118) individuals tested positive only for *Ureaplasma* spp. using the IST3 test, these individuals exhibited complete effectiveness with 100% sensitivity and no cases of intermediate or resistance to Erythromycin. Demonstrated very high sensitivity at 98.95% to Moxifloxacin, with only 1.04% intermediate response, and Telithromycin, with 1.04% of resistant cases. Achieving 97.91% sensitivity, Tetracycline had a small percentage of cases showing intermediate (1.04%) and resistant (1.04%) responses. And Levofloxacin showed effectiveness with 90.62% sensitivity, though resistance was present in 7.29% of cases and 2.08% were intermediate, suggesting it is largely effective but not without limitations (Table 3).

18 individuals (15.25%) out of 118 who tested positive for both *Ureaplasma* spp. and *Mycoplasma hominis* using the IST3 test. Since sensitivity results are separated at the coinfection scenario, Mycoplasma hominis achieved complete sensitivity at 100% for Tetracycline, and a high sensitivity rate of 88.89% for Levofloxacin, with 5.56% intermediate and 5.56% resistance, and Moxifloxacin, with 11.11% of cases showing resistance. Clindamycin showed a lower sensitivity rate of 83.33%, with 16.67% resistance, suggesting that it may be less reliable for *Mycoplasma* infections compared to other options. Although for Ureaplasma spp. Erythromycin achieved 100% sensitivity, with no cases of intermediate or resistance and 88.89% sensitivity and 11.11% resistance for Moxifloxacin, Tetracycline and Telithromycin. Levofloxacin showed moderate effectiveness against *Ureaplasma*, with 77.78% sensitivity and 22.22% resistance (Table 3).

**Table 3:** Antibiogram results for 4 positive cases of *Mycoplasma hominis*, 96 cases of *Ureaplasma spp.*, and 18 cases of coinfection with *Ureaplasma urealyticum* and *Mycoplasma hominis* as evaluated by the IST3 test. *Mycoplasma hominis* antibiogram results to Tetracycline, Levofloxacin, Moxifloxacin, and Clindamycin. *Ureaplasma spp.* antibiogram results to Tetracycline, Levofloxacin, Moxifloxacin, Erythromycin, and Telithromycin. For the coinfection cases, *Mycoplasma hominis* antibiogram with Tetracycline, Levofloxacin, Moxifloxacin, and Clindamycin, while *Ureaplasma urealyticum* with Tetracycline, Levofloxacin, Moxifloxacin, Erythromycin, and Telithromycin.

| Antibiotics | *Mycoplasm* | | | *Ureaplasm* | | |
|---|---|---|---|---|---|---|
| | S | I | R | S | I | R |
| Levofloxacin | 4 (100%) | 0 | 0 | 87 (90.62%) | 2 (2.08%) | 7 (7.29%) |
| Moxifloxacin | 4 (100%) | 0 | 0 | 95 (98.95%) | 1 (1.04%) | 0 |
| Tetracycline | 4 (100%) | 0 | 0 | 94 (97.91%) | 1 (1.04%) | 1 (1.04%) |
| Clindamycin | 4 (100%) | 0 | 0 | - | - | - |
| Erythromycin | - | - | - | 96 (100%) | 0 | 0 |
| Telithromycin | - | - | - | 95 (98.95%) | 0 | 1 (1.04%) |
| *Mycoplasm and Ureaplasm* | | | | | | |
| Levofloxacin | 16 (88.88%) | 1 (5.55%) | 1 (5.55%) | 14 (77.77%) | 0 | 4 (22.22%) |
| Moxifloxacin | 16 (88.88%) | 0 | 2 (11.11%) | 14 (77.77%) | 0 | 2 (11.11%) |
| Tetracycline | 18 (100%) | 0 | 0 | 16 (88.88%) | 0 | 2 (11.11%) |
| Clindamycin | 15 (83.33%) | 0 | 3 (16.66%) | - | - | - |
| Erythromycin | - | - | - | 18 (100%) | 0 | 0 |
| Telithromycin | - | - | - | 16 (88.89%) | 0 | 2 (11.11%) |

Legend: S: sensitive; I: intermediate; R: resistant; Absence of interpretation criteria.

## 1.4 Discussion

*Mycoplasma hominis* and *Ureaplasma spp.* are pathogens that colonize the urogenital tract of sexually active adults. They are often associated with opportunistic infections, meaning they can lead to diseases primarily in individuals with compromised immune systems or underlying health conditions. The treatment of infections caused by these pathogens is complicated by varying patterns of antibiotic resistance and sensitivity.

It has already been well-documented that *Mycoplasma* species are exhibiting rising global resistance to commonly used drugs like azithromycin, ciprofloxacin, and ofloxacin. This trend is notable across different regions, with studies in Europe and Asia reporting significant resistance rates to fluoroquinolones, tetracyclines and macrolides[16]. For instance, Belgium has recorded fluoroquinolone resistance rates as high as 31.3%, with similar patterns observed in Spain, Germany, and other European countries [4, 17]. The observed resistance compromises the efficacy of first-line treatments, which can exacerbate the challenge of managing such infections, particularly in the case of patients presenting co-infections or recurrent infections.

In the results presented by the Mycoplasma IST2 test, the highest resistance was observed in *Mycoplasma hominis* and *Ureaplasma urealyticum* to Ciprofloxacin and Ofloxacin, both of which are second and third generation fluoroquinolones, respectively. In our study, 75% of the individuals who tested positive for *Mycoplasma hominis* exhibited resistance to these antibiotics, indicating a significant limitation in treatment options for these patients. Similarly, *Ureaplasma urealyticum* showed 70.83% resistance to Ofloxacin and 84.16% resistance to Ciprofloxacin, suggesting that these fluoroquinolones are largely ineffective for treating infections caused by this species as well. This bacterial resistance to fluoroquinolones is associated with genetic alterations in DNA gyrase and/or the topoisomerase complex, which are the primary targets of these antibiotics. Such modifications can hinder the antibiotics' ability to bind effectively to their targets, thereby diminishing their therapeutic action and contributing to treatment failure [9, 18]. Furthermore, macrolides such as Erythromycin, Azithromycin, and Clarithromycin, two of which are derivatives of Erythromycin, exhibited a notable level of resistance among *Mycoplasma hominis* cases., with 25% resistance. This pattern highlights the growing challenge in managing infections caused by *Mycoplasma* and *Ureaplasma* species, as resistance to first-line treatment options can significantly limit therapeutic efficacy and complicate clinical outcomes.

Patients diagnosed through the Mycoplasma IST2 test who exhibited co-infections showed a marked increase in resistance frequencies, particularly with a significant prevalence of resistance to Erythromycin, reaching 60%. While Erythromycin and other macrolides generally demonstrate effective performance, our findings underscore a troubling trend among patients with co-infections [19, 20]. The presence of co-infections not only complicates the clinical management of these patients but also contributes to a more pronounced antibiotic resistance profile.

The frequency of positive cases for *Ureaplasma* spp. tends to be higher than that for *Mycoplasma hominis*, as observed in both the IST2 and IST3 tests. Among the 3.39% (4/118) of individuals who tested positive solely for *Mycoplasma hominis* via the IST3 test, none exhibited significant resistance to the antibiotics tested. In contrast, *Ureaplasma* spp. demonstrated a resistance rate of 7.29% and an intermediate response rate of 2.08% to levofloxacin, a fluoroquinolone. In cases of co-infection, a notable resistance of 22.22% was observed. Additionally, resistance to tetracyclines was documented in *Ureaplasma* at 1.04%, while in cases of co-infection,

resistance was recorded at 11.11%. This is a significant concern considering the drug's broad-spectrum antibiotic and have potent bacteriostatic activity against different pathogens [2].

However, it is important to note that tetracycline resistance in *Ureaplasma* spp. was higher in the IST2 tests within the context of coinfection, with a resistance frequency of 5% (1/20). The kit, though widely used, has a tetracycline concentration breakpoint (S ≤ 4µg/mL; R ≥ 8µg/mL) that conforms to CLSI standards for *Mycoplasma hominis*, but not for *Ureaplasma* spp., which should have a breakpoint of S ≤ 1µg/mL; R ≥ 2µg/mL, this mismatch leads to a significant underestimation of tetracycline resistance in Ureaplasma species [2, 14, 21].

The comparative analysis between the two diagnostic tests showed the disappearance of erythromycin resistance, which had been frequently noted in the coinfection scenario using the IST2 test. In IST2, erythromycin resistance was often overestimated in mixed cultures, however, in the IST3 test, the ability to test each species independently allowed for more accurate results, revealing that the erythromycin resistance seen in IST2 was largely a result of this methodological limitation [13]. These issues highlight the importance of using updated and validated methods like IST3. Additionally, the intrinsic antibiotic resistance of these organisms heightens the likelihood of failure in empirical treatment strategies for symptomatic urogenital infections [22].

Our results also demonstrated a significant variation in infection rates across age groups. Most cases occurred in individuals aged 38 to 47, accounting for 36.26% of the total (95/262), followed by the 28 to 37-year age group, which made up 32.44% (85/262). These findings suggest that *Mycoplasma* and *Ureaplasma* infections are most prevalent among sexually active middle-aged adults. Notably, the highest number of positive cases was observed in the 21-30 and 31-40 age brackets, further indicating a significant prevalence among younger, sexually active populations [23–25]. The concentration of cases within these age brackets highlights the importance of targeted public health interventions aimed at these populations, particularly in terms of awareness, prevention, and screening strategies. Further research is warranted to explore the underlying causes of these age-related trends and to develop tailored approaches for managing infections in these high-prevalence groups.

The study's sociodemographic data also highlights a striking 98.47% (258/262) of the diagnosed patients being women. This finding indicates that *Mycoplasma* and *Ureaplasma* infections disproportionately affect females within this population, aligning with existing research[24, 25]. The prevalence of *Mycoplasma* in the genital tract ranges from 5–20% in sexually active men and 40–80% in women, this higher incidence among sexually active women varies by region, country, and even within the same population, depending on ethnicity and socioeconomic status [2, 25, 26]. Additionally, although *Mycoplasma hominis* is part of the vaginal microbiota, it can become pathogenic in disturbed vaginal environments, acting opportunistically [6]. Similarly*, Ureaplasma urealyticum* is also present in the genital microbiota, with many of its infections in women occurring asymptomatically [4].

The findings from the antibiogram and sociodemographic data highlight the complex relationship between antimicrobial resistance and population characteristics. The significant resistance patterns among urogenital pathogens like *Mycoplasma* and *Ureaplasma*, especially in female patients, emphasize the urgent need for tailored diagnostic and therapeutic strategies. Additionally, resistance profiles can vary by region due to factors such as local antibiotic usage and healthcare practices, so continuous evaluation through specific testing is essential to address these variations. This approach not only optimizes patient outcomes but also preserves the efficacy of existing antimicrobial agents, enabling targeted treatment strategies that adapt to the evolving resistance landscape.

## Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Author contributions

VMF and SLSS had the conception and design of the study; SLSS acquired the data; VMF and SLSS analyzed the data; SLSS drafted the initial manuscript; SLSS, LVA and VMF revised the manuscript. All authors have approved the final manuscript.

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