

# Resistance Profile of Urogenital Mycoplasmas to Antibiotics: Comparative Study between the Mycoplasma IST2 and Mycoplasma IST3

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**How to cite this paper:** Akono, L., Gueguim, C., Sipewa, M.J.M., Assiene, S.D.O., Fezeu, M.N., Eboa, L.P., Ayangma, C.R. and Sone, L.H.E. (2024) Resistance Profile of Urogenital Mycoplasmas to Antibiotics: Comparative Study between the Mycoplasma IST2 and Mycoplasma IST3. *Advances in Microbiology*, 14, 578-588.

<https://doi.org/10.4236/aim.2024.1411040>

**Received:** September 10, 2024

**Accepted:** November 26, 2024

**Published:** November 29, 2024

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## Abstract

Acquired resistance of mycoplasmas to antibiotics constitutes a major health problem in the world in general and in Africa in particular. Despite the diversity of kits marketed, several of them evaluating the sensitivity of mycoplasmas to antibiotics continue to present resistance, in this case, the Mycoplasma IST2. In order to overcome this resistance, a new kit (Mycoplasma IST3) has been developed in accordance with the new Clinical and Laboratory Standard Institute (CLSI) recommendations. The objective of the study was to determine the resistance profile of urogenital Mycoplasmas to antibiotics using this new kit and to highlight cases of co-infections in comparison with the Mycoplasma IST2. Over a period of four months, one hundred and one (101) samples of urogenital secretions were collected (from sexually active men and women) and analyzed. Culture and antibiotic susceptibility testing were performed in a liquid medium using the Mycoplasma IST2 and Mycoplasma IST3 Tests according to the manufacturer's recommendations. Among the different samples analyzed, we noted a mycoplasma positivity rate of 71.29% with a predominance of infection in women, *i.e.* 86.12% compared to men 13.88%. *Ureaplasma spp* was the most encountered germ with a rate of 62.50% followed by cases of co-infections at 33.33% (*Usp*/*Mh*) and the least encountered was *Mycoplasma hominis* with a rate of 2.79%. We analyzed 25 samples, among which we had 4 co-infections, simultaneously using the two kits in the same patients. The distribution of cases between the two kits was equivalent.

We noted a significant rate of resistance to erythromycin 100% using Mycoplasma IST2. However, no resistance was observed in erythromycin with Mycoplasma IST3. Mycoplasma IST2 also showed resistance to fluoroquinolones, which was not the case for Mycoplasma IST3 which did not show any resistance to fluoroquinolones. Both kits showed resistance to tetracycline. The antibiotic sensitivity test using the Mycoplasma IST3 revealed a high rate of resistance to tetracycline, *i.e.* 57.14% and 91.67% for *Ureaplasma* spp and *Mycoplasma hominis* respectively. Resistance rates to other antibiotics were less than 25%. This study was able to demonstrate that Mycoplasma IST3 constitutes a better therapeutic choice compared to its predecessor Mycoplasma IST2, because it eliminated the biggest shortcoming of its predecessor.

## Keywords

Urogenital Infection, Mycoplasma IST2, Mycoplasma IST3, Antibiotic Resistance

## 1. Introduction

Urogenital mycoplasmas are one of the most common causes of gynecological infections [1] [2]. In Cameroon, the prevalence of 65% of urogenital mycoplasma has been reported among women hospitalized in Yaoundé [3], 38% among pregnant women in Douala [3] and 71.4% among HIV-infected women in Yaoundé [4]. Although commensal to the genital tract, these bacteria can become pathogenic in the event of abnormal proliferation in the genital tract or in association with other bacteria and cause several pathologies (pyelonephritis, endometritis, salpingitis), which are generally accompanied by complications such as miscarriages, prematurity, infertility and intrauterine infections [5]. Furthermore, they can be responsible for extra-genital infections, particularly in immunocompromised people [5]. These bacteria have no wall and are therefore resistant to families of antibiotics targeting the cell wall (Beta-lactams or polypeptides).

The most effective antibiotics against these bacteria are only those which disrupt the activity of DNA gyrase (fluoroquinolones) and those which inhibit the synthesis of prokaryotic proteins (Tetracycline and MLSK) [6]. In 1966, *in vitro* antibiotic sensitivity studies had already been carried out on these bacteria with evidence of antibiotic resistance [7]. Today, reports of mycoplasma resistance to antibiotics continue to increase each year [1] [8] [9]. Several kits capable of identifying and testing the sensitivity of mycoplasmas to antibiotics are on the market to facilitate the diagnosis of urogenital mycoplasmas. However, some of these kits have some limits, in this case the Mycoplasma IST2 Biomérieux kit which has limits in terms of the choice and concentrations of antibiotics tested for these germs due to its “all-in-one” system not allowing the identification and sensitivity testing independently for *Ureaplasma urealyticum* and *M. hominis*, particularly in the case of co-infections, knowing that *M. hominis* has natural resistance to 14- and

15-membered macrolides [10].

Previous studies demonstrated acquired resistance growth for tetracycline, macrolides and fluoroquinolones due to mutations in target genes [10]. To correct this, a new Mycoplasma IST3 Biomérieux kit allowing an independent assessment of antibiotic sensitivity with new choices and concentrations of antibiotics for each species (*M. hominis* and *Ureaplasma* spp) has been set up in accordance with the new Clinical and Laboratory Standard Institute recommendations. Thus, determining the resistance profile of urogenital Mycoplasmas to antibiotics using this new kit and highlighting cases of co-infections in comparison with the Mycoplasma IST2 Biomérieux kit was the objective assigned to this study.

## 2. Materials and Methods

### 2.1. Collection and Transport of Samples

#### 2.1.1. Collection

The 101 urogenital secretions collected using swabs were obtained after compliance with sampling requirements and disinfection of the sampling site using dakin, thus avoiding contamination of the samples.

#### 2.1.2. Transport

After sampling, the swabs were clearly labelled in their case and immediately sent to the bacteriology department for analysis.

### 2.2. Bacteriological Analysis

#### 2.2.1. Principle and Composition of the Medium

These kits are used for clinical isolation culture and antibiotic susceptibility testing of *Ureaplasma* spp and *Mycoplasma hominis*. The mycoplasma culture media (IST2 and IST3) contain a basic mycoplasma broth, peptone, yeast extract, an indicator (phenol red), a mixture of antibiotics, growth factors, urea, arginine and other substances. As *U*spp and *M*h grow, the alkaline substances generated by the decomposition of urea and arginine cause an increase in the pH value. The medium will change colour from yellow to red. The media contain a bacteriostatic agent that can inhibit the growth of bacteria and fungi (erythromycin for *Mycoplasma hominis*, lincomycin for *Ureaplasma* spp and an antifungal agent) in the sample.

#### 2.2.2. Enumeration, Identification and Antibiotic Susceptibility Testing

These tests are carried out aseptically, after inoculation of the secretions with the reagent combination (R1 and R2) and vortex mixing, 55 µL of urea-arginine broth from the IST2 and IST3 galleries are immediately distributed in each of the 22 test cups for IST2 and the 25 test cups for IST3, then covered with two drops of paraffin oil to create the anaerobiosis required for mycoplasma culture. The different galleries were then incubated at 37°C and read after 24 hours for *Ureaplasma* spp and 24 - 48 hours for *Mycoplasma hominis*.

These cups enabled us to determine whether the mycoplasma titre in the sample

was equal to or greater than a threshold set at  $10^4$  CFU for the IST2 kit and greater than or equal to different thresholds:  $10^3$ ,  $10^4$ ,  $10^6$  CFU/ml (colony-forming unit) for the IST3 kit. The differentiation of antibiotics for each germ meant that the sensitivity test could be read more accurately as either resistant or sensitive. No intermediates.

### 2.3. Data Processing and Analysis

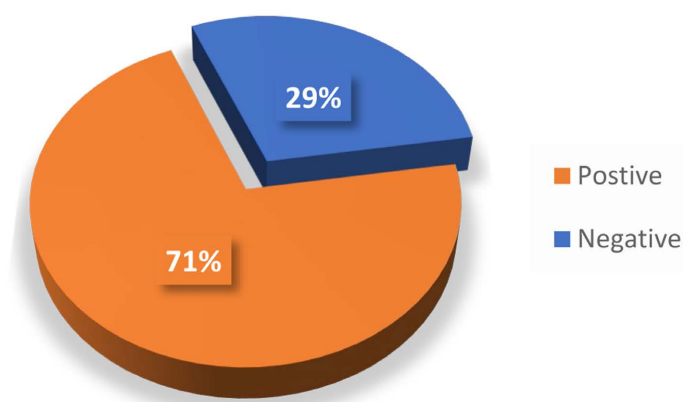
The data were collected, processed and analysed using Excel software. The results were presented in table, graph or narrative form.

## 3. Results

Apart from antibiotic resistance, our study took into account certain parameters (age and sex) influencing mycoplasma infections. For this study, we established the resistance profile of co-infections using the Mycoplasma ITS3 kits and its predecessor Mycoplasma IST2 Biomérieux simultaneously for the same samples in order to clearly visualise the behaviour of the two kits in the case of co-infections. Subsequently, this antibiotic resistance profile was established for Mycoplasma IST3 Biomérieux alone.

### 3.1. Prevalence of Urogenital Mycoplasma Isolated Using the Biomérieux Mycoplasma IST3 Kit

**Figure 1** shows that of the 101 samples recorded and analysed, the positivity rate was high at 71.29% (72/101).



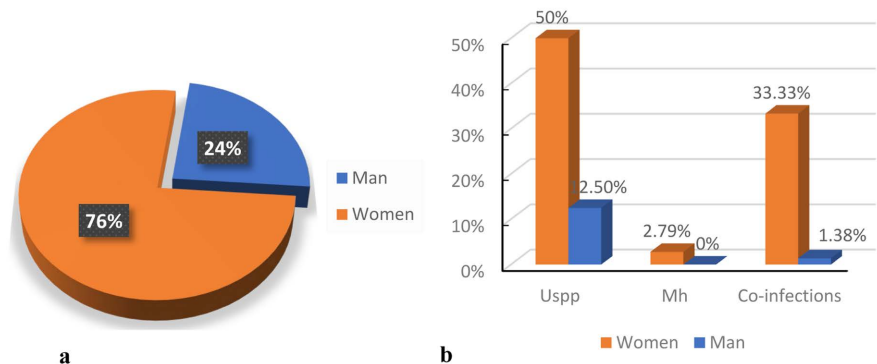
**Figure 1.** Prevalence of urogenital mycoplasma infection.

### 3.2. Prevalence and Distribution of Mycoplasma Infections by Sex

When looking for urogenital mycoplasmas, we were able to record a rate of 76% (77/101) in women compared with 24% (24/101) in men (**Figure 2(a)**), with a female/male ratio of 3.20.

**Figure 2(b)** shows that mycoplasma was more prevalent in women than in men, with *Ureaplasma* spp infection predominating at 50% (36/72) in women and 12.50% (9/72) in men. A very low *M. hominis* rate of 2.79% (2/72) was also

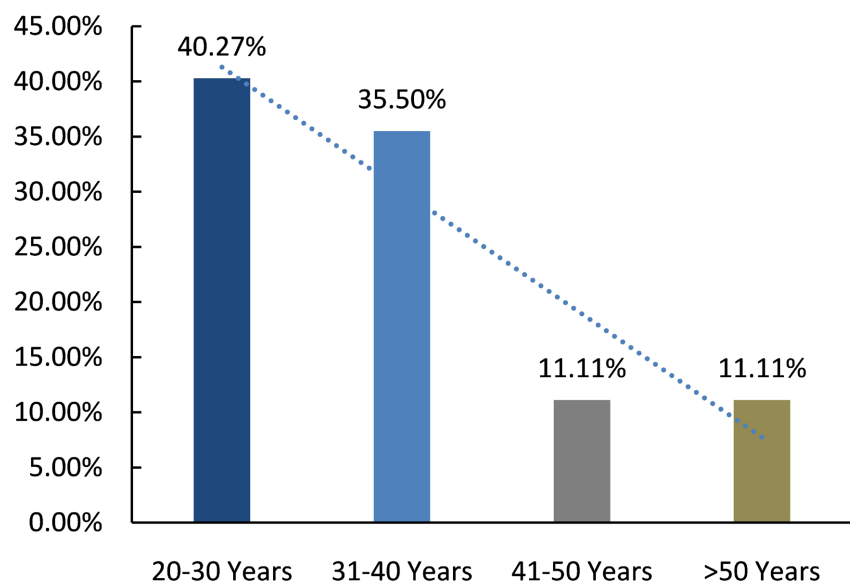
observed in women. The rate of co-infections was 33.33% (24/72) and 1.38% (1/72) in women and men respectively.



**Figure 2.** (a) Prevalence of mycoplasma test requests according to sex; (b) Distribution of urogenital mycoplasma infections according to sex.

### 3.3. Prevalence of Infection According to Age

**Figure 3** shows that young people, particularly those aged 20 - 30, were the most infected, followed by those aged 31 - 40.



**Figure 3.** Prevalence of infection according to age.

### 3.4. Mycoplasma IST2/ Mycoplasma IST3 Co-Infection States

A total of 25 samples from the same patients were simultaneously tested using the two kits. We observed an almost equivalent distribution for the two kits (Mycoplasma IST2 and Mycoplasma IST3) as shown in **Table 1**. However, we noted a significant difference between the two kits in the antibiotic susceptibility test results for these co-infections, particularly macrolides (Erythromycin) (**Table 2**).

**Table 1.** Distribution of mycoplasma cases according to infection category.

Kits	Mono-infection	Negative cases	Co-infections	Total
<b>Mycoplasma IST2</b>	12	09	04	25
<b>Mycoplasma IST3</b>	12	09	04	25

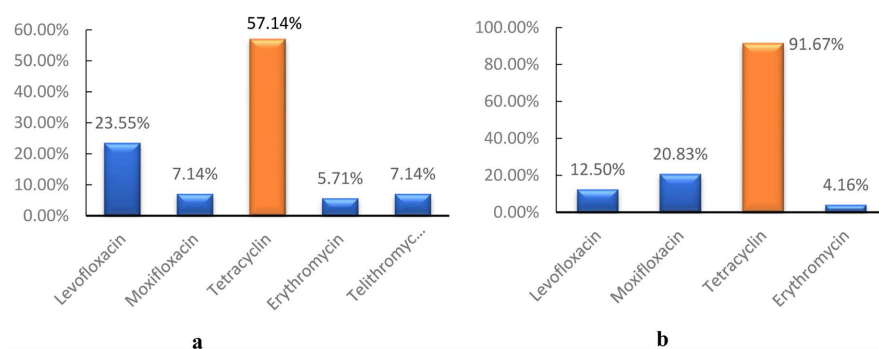
**Table 2.** Mycoplasma IST2/Mycoplasma IST3 co-infection sensitivity test.

Antibiotics	Kit IST2				Kit IST3				Families				
	Uu/Mh				Uspp					Mh			
	1	2	3	4	1	2	3	4					
<b>Doxycycline</b>	S	S	R	I	R	R	R	R	R	R	R	R	Cyclines
<b>Tétracyclin</b>	R	I	R	R	R	R	R	R	R	R	R	R	Cyclines
<b>Erythromycin</b>	R	R	R	R	S	S	S	S					M 1 G
<b>Josamycin</b>	I	S	S	S									M 2 G
<b>Clarithromycin</b>	R	R	R	R									M 2 G
<b>Azithromycin</b>	R	R	R	R									M 2 G
<b>Clindamycin</b>									S	S	S	S	Lincosamides

**Legend:** Uu = *Ureaplasma Urealyticum*; Mh = *Mycoplasma hominis*; Uspp = *Ureaplasma spp*; S = Sensible; R = Resistant; I = Intermediate; M1G = Macrolides 1st Generation; M2G = Macrolides 2nd Generation.

### 3.5. Antibiotic Resistance Profile

This study revealed that *Ureaplasma spp* were resistant to tetracyclines 57.14% (40/70) followed by levofloxacin 23.55% (16/70) and more rarely moxifloxacin 7.14% (5/70), telithromycin 7.14% (5/70) and erythromycin 5.71% (4/70), in contrast to *Mycoplasma hominis*, which showed strong resistance to tetracyclines 91.67% (22/24), followed by moxifloxacin 20.83% (5/24) and resistance of less than 15% to levofloxacin and clindamycin (as shown in **Figure 4**).

**Figure 4.** (a) Resistance profile of *Ureaplasma spp* (Mycoplasma IST3 kit); (b) Resistance profile of *Mycoplasma hominis* (Mycoplasma IST3 kit).

## 4. Discussion

In the present study, identification and antibiotic susceptibility testing were performed on vaginal and urethral swabs using the Mycoplasma IST3 Biomérieux and Mycoplasma IST2 Biomérieux kits.

The positivity rate for mycoplasma using the Biomérieux Mycoplasma IST3 kit was 72%. This rate is higher than that obtained in a study in Yaounde which was 65% [3] and higher than the rate obtained by Guindo, Zheng *et al.* in different geographical areas which were respectively 63.5%, 18.6%, 38.39% [11] [12]. These variations may be due to the fact that these studies were carried out using Mycoplasma IST2 Biomérieux which has a pathogenicity threshold set at  $10^4$  UFC/ml [13], unlike Mycoplasma IST3 which has a pathogenicity threshold set at  $10^3$  UFC/ml [14]. For Ureaplasma, mycoplasmas (*Ureaplasma* spp) are considered to be pathogenic from  $10^3$  UFC/ml. The epidemiology of Mycoplasma infection also varies according to the population studied in different geographical areas [15].

The prevalence of mycoplasma was 50% and 12.50% for *Ureaplasma* spp in men and women respectively, and 2.79% and 0% for *Mycoplasma hominis* in men and women respectively. These rates confirm the statements of Djifack Tadongfack *et al.*, according to whom the prevalence of mycoplasma infection (*U*spp and *M*h) in asymptomatic sexually active adults varies from 40 to 80% in women and 5 to 20% in men, with *Mycoplasma hominis* rates always lower than *Ureaplasma* spp. The incidence of each germ (*U*spp and *M*h) is slightly lower in the urethra [16] [17]. The most common age groups were 20 - 30 and 31 - 40, with infection rates of 40.27% and 37.50% respectively. This is in line with global reports which classify the 15 - 35 age group as the most affected (78.39%). This may be justified by the high-risk sexual activity in these age groups. Genital carriage of these microorganisms is directly related to the number of sexual partners in adults [18].

In the co-infection states established using the Mycoplasma IST3 Biomérieux and Mycoplasma IST2 Biomérieux kits, we were able to note a high rate of resistance to erythromycin using the IST2 kit, unlike the IST3, which showed no resistance to erythromycin, just susceptibility to *Ureaplasma* spp. Unfortunately, this sensitivity to *Ureaplasma* spp cannot be visualised with the Biomérieux Mycoplasma IST2. This is particularly due to the structural difference between the two kits.

The inability of Mycoplasma IST2 to assess the two species independently. Indeed, *Mycoplasma hominis* has a natural resistance to 14C and 15C macrolides (Erythromycin, Azithromycin) [19], this resistance to erythromycin observed with the Biomérieux Mycoplasma IST2 may be that of *Mycoplasma hominis* naturally resistant to erythromycin. This blurs the sensitivity of *Ureaplasma* spp observed in the Biomérieux Mycoplasma IST3 kit, which is able to assess both germs independently. High rates of resistance were also observed among the fluoroquinolones in the Mycoplasma IST2, which may be due to the fact that most of these molecules have been on the market for some time. These drugs are generally prescribed. They are also within the reach of the general public (self-medication),

which is generally the cause of acquired resistance to bacteria, hence the need to test new molecules such as the fluoroquinolones in *Mycoplasma* IST3 Biomérieux, which have not shown any resistance. Also, inappropriate concentrations of antibiotics tested in Biomérieux *Mycoplasma* IST2 may also be the cause of these resistances.

In addition, high rates of resistance to tetracycline were observed in strains of *Ureaplasma* and *M. hominis*; 57.41% and 91.67% respectively. These rates are much higher than those obtained in Dschang, Cameroon, where Mh and Uspp strains showed cyclin resistance of 24.4% and 6.5% respectively [18]; another study carried out in Bamako showed cyclin resistance of 36% and 71% for *Ureaplasma* and *hominis* respectively [11]. In Gabon, resistance rates varied between 37.2% for Uspp, 57.1% for Mh and 61.1% for co-infections [20]. However, these data are comparable to those obtained in Abidjan, where total cyclin resistance was 92% for *M. hominis* and 52% for *Ureaplasma* spp [21]. As most of these studies were carried out using the Biomérieux *Mycoplasma* IST2 Kit, all these variations may be due to the structural difference between the Biomérieux *Mycoplasma* IST2 and the Biomérieux *Mycoplasma* IST3, which has the capacity to evaluate the two mycoplasma species independently thanks to a different choice and concentration of antibiotics for each species. Geographical differences may also influence these results [15]; however, tetracyclines are the agents most widely used to control mycoplasma infection of the urogenital tract in adults [22] [23]. The development of tetracycline resistance in mycoplasmas and in some cases will be associated with the acquisition of the tet(M) determinant located at the Tn916 transposon [24]. The transposon codes for the TetM protein, protecting ribosomes from the effects of tetracyclines.

Resistance rates to fluoroquinolones in *Ureaplasma* spp ranged from 7.14% to 23.55% for moxifloxacin and levofloxacin respectively. Between 12.50% and 20.83% for *Mycoplasma hominis*. These resistance rates are much lower than those obtained in three studies in Africa using the Biomérieux *Mycoplasma* IST2 [11] [19] [20]. In the study conducted in Gabon, this rate varied from 97.20% to 100% for *Ureaplasma* spp and 95.20% to 96.80% for *Mycoplasma hominis* [20]. In Dschang, this rate varied from 5.30% to 13.0% for *Ureaplasma* strains, 10 to 20% for *Mycoplasma hominis* strains [19]; and in Bamako, from 87.50 to 96.70 for *Mycoplasma hominis* and *Ureaplasma urealyticum* respectively [11]. Resistance to quinolones results essentially from the selection of mutations in DNA gyrase or topoisomerase IV following the widespread use of these molecules. Most of these molecules have been prescribed since childhood because of their mild side effects, which is thought to have reduced the susceptibility of mycoplasmas to fluoroquinolones.

The rate of resistance to clindamycin was low. Clindamycin is a new antibiotic from the Lincosamide family tested in the Biomérieux *Mycoplasma* IST3. This low rate of resistance must be due to the fact that it is a new antibiotic that is not yet widely used. Uspp resistance rates were 5.71% to erythromycin and 7.14% to

telithromycin. The rate of *Ureaplasma* resistance to erythromycin in this study is much lower than those reported by authors ranging from 33.3% to 97.8% [25] [26]. This huge difference could be linked to the new antibiotic concentrations tested in the Biomérieux Mycoplasma IST3, which are in line with the new CLSI recommendations. Telithromycin is a new antibiotic tested in Mycoplasma IST3 for *Ureaplasma* spp. It is a macrolide derivative to which *M. hominis* has natural resistance.

## 5. Conclusion

The results of this study showed that 71.29% of women were positive for urogenital mycoplasma infections, compared with 28.71% of men. The most common germ was *Ureaplasma* spp (62.50%), followed by co-infections (*U*spp/*Mh*) (34.71%) and *Mycoplasma hominis* (2.79%). In the case of co-infections, we found significant resistance to erythromycin and fluoroquinolones using the IST2 kit, unlike the IST3 kit, which showed no resistance to these antibiotics. However, these two kits were resistant to tetracycline, *i.e.* 57.14% and 91.67% to *U*spp and *Mh* respectively. Bacterial resistance to antibiotics is an unavoidable but controllable fact.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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