

# Trends in the prevalence and antibiotic resistance of *Ureaplasma urealyticum* strains isolated during sperm culture at the biology laboratory of the Institut National d'Hygiène Publique (INHP) in Abidjan (Côte d'Ivoire), 2018 to 2021

## ABSTRACT

**Aims:** *Mycoplasmas* are bacteria of the urogenital tract often associated with various infections and infertility. In terms of treatment, acquired resistance to antibiotics has been reported. The aim of this study was to investigate changes in the prevalence and antibiotic resistance of *Ureaplasma urealyticum* strains isolated during sperm culture, in order to help update therapeutic protocols for the treatment of *U. urealyticum*-associated infections

**Place and Duration of Study:** These were men received at the INHP from January 2018 to December 2021 for sperm culture

**Methodology:** This is a retrospective study in which the culture results of 907 semen samples received in the laboratory between 2018 and 2021 were analysed. Identification, indicative counts and antibiotic susceptibility testing of *U. urealyticum* were performed using the Biosynex Mycoplasma kit.

**Results:** 283 strains of *U. urealyticum* were isolated between 2018 and 2021, with an average prevalence of 31.2%. In 2018, only minocycline, josamycin and roxithromycin were active on all the strains tested, while fluoroquinolones and clindamycin showed high levels of resistance, with rates of 75.9% and 86.1% respectively. From 2019 onwards, resistance spread to all antibiotics, with increasing levels of resistance, with the exception of minocycline, which remained active on all strains. The highest resistance rates were observed in 2021 and were 74.6% and 96.6% for pristinamycin and clindamycin respectively.

**Conclusion:** Despite the high level of resistance, minocycline and josamycin have shown relatively stable antibacterial activity and could be proposed as the molecules of choice for the treatment of *U. urealyticum* infections. Closer monitoring of the development of antibiotic resistance in these bacteria is therefore necessary.

*Keywords: infertility, human reproduction, bioresistant, germs, Ivory Coast*

## 1. INTRODUCTION

*Ureaplasma urealyticum* is a commensal bacterium of the genitourinary tract with a carriage rate of up to 80% in sexually active women [1]. It is an opportunistic pathogen, of the mycoplasma family, that can cause persistent non-gonococcal urethritis [2], prematurity, postnatal endometritis, chorioamnionitis. and spontaneous abortions [3; 4].

*U. urealyticum* has also been associated with prostatitis, epididymitis, chronic urethrocystitis and infertility [5; 6]. Therapeutically, potentially active antibiotics recommended in the treatment of mycoplasma infections belong to the cyclin, macrolide and related families and fluoroquinolones [7]. In recent years, acquired resistance to these antibiotics has been reported by several authors, making it difficult to implement standard treatments [8,9,10]. Controlling the spread of bacterial resistance requires country to have regular and reliable data on the frequency and characteristics of this resistance [11]. semen, analysis also plays an important role in the assesment of male infertility highlightinga man's fertility capacity [12]. In addition, to detecting sperm abnormalities, it can be used to investigate associated microbial aetiologies and the antibiotic resistance profile. At the National Institute of Public Hygiene (INHP), spermiology accounts for approximately 10% of the examinations carried out in the hygiene laboratory. In 2017, *U. urealyticum* was implicate in 18% of positive sperm cultures with high rate of resistance to several antibiotics including fluoroquinolones (96.3%), pristinamycin (77.8%) and clarithromycin (48.1%). The aim of this study was to investigate changes in the prevalence and antibiotic resistance of *Ureaplasma urealyticum* strains isolated during sperm culture in order to help update therapeutic protocols for the treatment of *Ureaplasma urealyticum* associated infections

## 2. material and methods

### 2.1 Type and scope of the study

This was a retrospective study which involved analyzing the results of sperm culture performed at the INHP biology laboratory.

### 2.2 Study population

These were men received at the INHP from January 2018 to December 2021 for sperm culture.

### 2.3 Material

The biological material consisted of sperm collected in situ. The Biosynex Mycoplasma Kit was used for research, identification and antibiotic susceptibility testing.

### 2.4 Methods

#### **2.4.1. Culture and identification**

The Biosynex Mycoplasma kit is based on the culture and biochemical reactions that cause the colour indicator to change colour. It combines a broth adapted to the culture of urogenital mycoplasmas (pH, substrates and a combination of several growth factors) and enables *Ureaplasma urealyticum* and *Mycoplasma hominis* to be cultured, identified, counted and tested for antibiotic sensitivity. The presence of specific substrates (urea for *Ureaplasma urealyticum* and arginine for *Mycoplasma hominis*) and a pH indicator (phenol red) means that, in the event of a positive culture, a change in colour linked to an increase in pH can be observed. The result was interpreted as positive if an orange to red colour appeared, and the infection threshold was set at a value greater than or equal to 10<sup>4</sup> CFU (Colony Forming Unit), which defines colonisation for a titre of less than 10<sup>4</sup> CFU. Cups were read after 24 hours and then 48 hours'incubation at 36°C ± 2°C.

### 2.4.2. Antibiotic sensitivity study

It was based on the presence or absence of bacterial growth in the different wells containing the antibiotics, with growth being reflected by the change in pH indicator. The molecules tested belonged respectively to the cyclin (tetracycline, minocycline), macrolide and related (josamycin, erythromycin, clindamycin, pristinamycin, roxithromycin, clarithromycin) and fluoroquinolone (ciprofloxacin, ofloxacin) families of antibiotics.

### 3. results

Nine hundred and seven (907) semen samples were analysed. The mean age of the subjects was 40.6 years, with extremes of 21 and 74 years. Two hundred and eighty-three (283) samples were infected with *Ureaplasma urealyticum*, representing a positivity rate of 31.2% (283/907). The distribution of *U. urealyticum* infections is given in Table 1.

**Table 1.** Breakdown of *U. urealyticum* infections into mono- and co-infections

Bacterial species	Number (%)
<i>Ureaplasma urealyticum</i>	119 (42.0)
<i>Chlamydia trachomatis</i> + <i>Ureaplasma urealyticum</i>	129 (45.6)
<i>Ureaplasma urealyticum</i> + <i>Gardnerella vaginalis</i>	5 (1.8)
<i>Chlamydia trachomatis</i> + <i>Ureaplasma urealyticum</i> + <i>Gardnerella vaginalis</i>	30 (10.6)
Total	283 (100)

Mono-infections with *U. urealyticum* accounted for 42%. Co-infections, mainly with *Chlamydia trachomatis*, were reported (45.6%). The majority of these infections were found in subjects aged between 31 and 40 years in figure 1.

**Fig. 1.** Breakdown of subjects infected with *Ureaplasma urealyticum* by age group

The prevalence of *U. urealyticum* by year is shown in Table 2, with high prevalences averaging 31.2%.

**Table 2.** Prevalence of *U. urealyticum* by year

Year	Number of samples	Positive culture	
		Effective	Percentage
2018	255	79	31
2019	205	73	35.6
2020	281	72	25.6
2021	166	59	35.5
Total	907	283	31.2

With regard to the sensitivity of *U. urealyticum* strains to antibiotics, the results are presented in Table 3.

**Table 3.** Antibiotic resistance rates of *Ureaplasma urealyticum* strains

Antibiotics (filler)	Resistance rate
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	2018 (n=79) N (%)	2019 (n=73) N (%)	2020 (n=72) N (%)	2021 (n=59) N (%)
Fluoroquinolones				
Ciprofloxacin (2 mg/l)	60 (75.9)	69 (94.5)	66 (91.7)	51 (86.4)
Ofloxacin (4 mg/l)	60 (75.9)	69 (94.5)	65 (90.3)	51 (86.4)
Cyclins				
Tetracycline (2 mg/l)	13 (16.5)	11 (15.1)	14 (19.4)	11 (18.6)
Minocycline (8 mg/l)	0 (0)	0 (0)	1 (1.4)	3 (5.1)
Macrolides and related				
Josamycin (8 mg/l)	0 (0)	9 (12.3)	1 (1.4)	5 (8.5)
Erythromycin (16 mg/l)	1 (1.3)	3 (4.1)	5 (6.9)	2 (3.4)
Clindamycin (0.5 mg/l)	68 (86.1)	70 (95.9)	68 (94.4)	57 (96.6)
Pristinamycin (2 mg/l)	30 (38)	27 (37)	50 (69.4)	44 (74.6)
Roxithromycin (4 mg/l)	0 (0)	4 (5.5)	3 (4.2)	2 (3.4)
Clarithromycin (4 mg/l)	2 (2.5)	20 (27.4)	9 (12.5)	15 (25.4)

In 2018, minocycline, josamycin and roxithromycin were active on all the strains tested, while other molecules such as pristinamycin, fluoroquinolones and clindamycin showed high levels of resistance, with rates of 38%, 75.9% and 86.1% respectively. From 2019 onwards, resistance spread to all antibiotics, with increasing levels of resistance, with the exception of minocycline, which remained 10,6% (30) 45,6% (129) 34,6% (98) 8,1% (23) 0,71% (2) 0,35 (1) 0% 5% 10% 15% 20% 25% 30% 35% 40% 45% 50% [21-30] [31-40] [41-50] [51-60] [61-70] [71-80] active on all strains. The highest resistance rates were observed in 2021 and were 74.6% and 96.6% for pristinamycin and clindamycin respectively.

#### 4. DISCUSSION

The mean age of the subjects in this study was 40.6 years and is relatively close to the 39 years observed in the studies by [13] and [14]. The subjects in the study by [15] in Tunisia in 1987 and 16 in China in 2019 were younger, aged 26-35 and 21-30 respectively, in line with the literature which suggests that *U. urealyticum* carriage affects the young and sexually active population [1]. This trend was also described by [17] in China. The often advanced age of the patients in our study could be explained by the delay in consulting a doctor due to the myth that women are solely responsible for the couple's infertility. The prevalence of *U. urealyticum* infection was relatively stable over the study period, with a mean value of 31.2%, close to that reported by [17] (30.8%) but lower than those reported by [14] and [16], which were 1.6% and 20.3% respectively. These differences could be linked to detection methods and variations from one country to another. This high rate could be explained by low adherence to global health sector strategies to combat HIV, hepatitis and sexually transmitted infections [18]. Mycoplasmas are bacteria without a cell wall, which gives this family of bacteria a natural resistance to antibiotics acting on the bacterial wall. A study of the behaviour of isolated strains of *U. urealyticum* towards the antibiotics tested showed relatively low resistance to minocycline in the case of cyclins and josamycin, and to erythromycin and roxithromycin in the case of macrolides and related agents. The rates observed with minocycline ranged from 1.4% to 5.1% and 15.1% to 19.4% for tetracycline. [16] found a minocycline resistance rate of 1.53%, while [19] in 2013, and [20] in 2017, reported higher rates for tetracycline of 29.5% and 26.2% respectively. These differences could be attributed to the frequency of use of these molecules, which varies from one country to another. In our study, the highest rates of resistance in *U. urealyticum* strains to josamycin, erythromycin and roxithromycin were 12.3%, 6.9% and 5.5% respectively. Our results differed from those of [20], for whom 23% and

47.5% of strains were resistant to josamycin and erythromycin respectively, while for [18], 26.4% of strains were resistant to erythromycin. Wei-wei Zheng et al. reported a roxithromycin resistance rate of 12.9%, higher than ours. Resistance to pristinamycin increased throughout the study period, with rates ranging from 38% to 74.6% in 2021, which is contrary to the results obtained in the Guindo and Sogodogo study, with rates of 13.1% and 0% respectively. With regard to fluoroquinolones (ofloxacin, ciprofloxacin), the strains showed high levels of resistance, with rates ranging from 75.9% to 94.5%; although these rates were close to those of [20], they were lower than those of [19] in fact, for these authors, the rates of resistance to ciprofloxacin were 96.7% and 65.9% respectively. This high level of resistance to fluoroquinolones could be attributed to the selection pressure exerted by "overuse" and misuse of these antibiotics in other specialities [21].

## 5. Conclusion

The results showed a high prevalence of *U. urealyticum* carriage, indicating the need to step up the fight against STIs in Côte d'Ivoire. The study also revealed a high level of resistance to certain molecules, such as fluoroquinolones (ofloxacin, ciprofloxacin), raising the alarm about the over-prescription of these antibiotics. Despite the high level of resistance, minocycline and josamycin have shown relatively stable antibacterial activity and could be proposed as the molecules of choice for the treatment of *U. urealyticum* infections. Closer monitoring of the development of antibiotic resistance in these bacteria is therefore necessary.

## Competing interests

Authors have declared that no competing interests exist.

## Consent

As per international standards or university standards, respondents' written consent has been collected and preserved by the author(s).

## Ethical approval

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

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