

Prevalence and Antibiotic Resistance of *Ureaplasma urealyticum* in Sperm Cultures: A Retrospective Analysis", Abidjan, Côte d'Ivoire

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: *U. urealyticum*, Prevalence, Antibiotic resistance, Abidjan, Côte d'Ivoire

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, highlighting a man's fertility capacity [12,22-24]. In addition to detecting sperm abnormalities, it can be used to investigate associated microbial etiologies 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 sperm ejaculates obtained were analyzed immediately. 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 suspension for culture was obtained by adding 100 µl of sperm to 1.3 ml of dilution liquid. After homogenization, the suspension was distributed to the cups at a rate of 50 µl per cup. After culture, urease from *U. urealyticum* breaks down urea (specific substrate), releasing NH₃. *Mycoplasma hominis* arginase breaks down arginine (specific substrate), releasing NH₃. Released NH₃ increases the pH of the liquid medium. The result is assessed by the color change of the pH indicator. If the mycoplasma present is sensitive to the antibiotics incorporated in the cups, enzyme activity is blocked and there is no color change. In male subjects, the pathogenicity criteria for *U. urealyticum* are as follows: ≥ 10⁴ CCU/ml (Color Changing Unit) for a urogenital swab and ≥ 10³ CCU/ml for the first urine stream. 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 whether or not the pH indicator turned red in the different wells containing the antibiotics, with growth being reflected by the pH indicator turning red. 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. In cups where *U. urealyticum* is sensitive to the antibiotic, the color of the medium remains unchanged at dilution 10⁻⁴. Conversely, when the bacterium is resistant to the antibiotic, its growth is highlighted by the pH indicator turning red at dilution 10⁻⁴.

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			
	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 prevalence of *U. urealyticum* was high in men received at the hygiene laboratory of the At the National Institute of Public Hygiene as part of the couple's infertility work-up. Antibiotic susceptibility testing showed a high level of resistance to ofloxacin and ciprofloxacin. This very worrying situation highlights the need to sound the alarm about over-prescription of these antibiotics, most often as first-line treatment for certain infections, but also about self-medication in developing countries such as Côte d'Ivoire. Minocycline and josamycin, with their relatively stable activity, could be proposed as the molecules of choice for the treatment of *U. urealyticum* infections. Closer monitoring of the evolution of antibiotic resistance in these bacteria is therefore essential.

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).

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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