



Epidemiology of The Cultivable Urogenital Mycoplasmas in The Center of Morocco

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Abstract

Urogenital mycoplasma infection can cause health problems and psychological seriousness of the fact that low infections, usually asymptomatic, can develop into serious complications with risks on fertility. Their importance in Morocco is not well understood due to the irregularity of their diagnosis.

Goal: Contribute to the epidemiology of these infections in our region.

Patients and methods: Cross-sectional study where the data collection is spread over three months, carried out at the bacteriology laboratory of military hospital of Meknes. Detection of Mycoplasma has been based on the liquid culture (Mycoplasma Test™ All Diag, France).

Results: 96 patients were explored. The m/f sex ratio was 1.6. The median age was 33.5 years. The most common complaint was infertility. The prevalence of *Ureaplasma urealyticum* (Uu) was 28.8% among men and 37.84% for women, and respectively 5.08% vs 16.22% for *Mycoplasma hominis* (Mh). The coinfection rate Uu + Mh was 5.21%. No factor predisposing statistically associated with the infection were found. The resistance of Uu was close to 10% against doxycycline and azithromycin vs 0% and 83% for Mh and 0% against levofloxacin for both germs.

Conclusion: Our results are comparable to those recorded in the national and international literature. The absence of factor favoring statistically linked to infection by Mycoplasma is probably related to the commensal character of these bacteria. Conventional methods of diagnosis like that used in our study are acceptable performance.

Keywords: Antibiotic Resistance; Infertility; *Mycoplasma hominis*; *Ureaplasma urealyticum*

Introduction

Urogenital mycoplasmas (MUG), including *Ureaplasma urealyticum* (Uu) and *Mycoplasma hominis* (Mh) are frequently isolated from the reproductive tract of healthy women. Their colonization rates are around 40% for Uu and 1.5% to 11% for Mh [1]. Colonization by these bacteria is statistically associated with several contributing factors, including young age, low socioeconomic status, sexual activity and multiple partners [2]. The pathogenic role of both MUGs is unanimously accepted. Indeed, Mh has been isolated from amniotic fluid in women with severe chorioamnionitis and premature deliveries and from the low urogenital tract (TUG). In addition, anti-Mh antibodies were detected at higher rates in infertile women with pelvic inflammatory disease, compared with controls. Similarly, Uu was more frequently isolated from spontaneous abortions and from premature infants compared to normal children, and with a greater prevalence in infertile women. The bacterial vaginosis materialized by *Gardnerella vaginalis* (Gv) is also characterized by a frequent association with MUG [3].

In terms of diagnosis, given their status as commensals, tests involving agar culture or broth remain recommendable because of the need for the quantitative aspect to incriminate them. They do not allow a priori to distinguish Uu from *Ureaplasma parvum* (Up) but allow tests of sensitivity to antibiotics. The exception is *Mycoplasma genitalium* (Mg), very tedious, requiring the use of nucleic acid amplification techniques (NAAT) [4].

The objectives of this work are to search for culturable MUGs, to estimate their prevalence at the Moulay Ismail Military Hospital in Meknes (HMMI) and to highlight the epidemiological, demographic and clinical characteristics of the population studied with the search for possible risk and determine the sensitivity of identified MUGs to antibiotics.

Patients and Methods

This is a cross-sectional study of patients of both sexes for a period of 3 months, from September 1, 2015 to November 30, 2015. Included were 96 patients who were externally followed or hospitalized and who received a medical prescription. for genital specimens for cytobacteriological study. Whether the search for MUGS is explicit or not, systematic screening has been performed on all harvested samples. A patient sheet has been prepared to supplement and standardize information useful for statistical and epidemiological analysis. The urogenital samples (urethral, first urine shot (PJU), sperm) were made at the microbiology specimen room and the vulvovaginal and cervical specimens were taken at the gynecology-obstetrics department of our formation. The test used is manufactured by All Diag, called "Mycoplasma Test®", is intended for culture, identification, indicative numeration and determination of antibiotic sensitivity of *M. hominis* and *U. urealyticum*.

Results

During the 3-month period, 96 patients were seen. The vast majority (91/96 or 94.8%) are followed externally. They were divided into 37 patients (sex f: 38.54%) and 59 patients (sex m: 61.46%), i.e. a sex ratio M / F of 1.6. The overwhelming majority of patients included were married (n = 88, or 91.67%). The average age was 34.49 +/- 8.89 years, with a median of 33.5 years (range: 12 to 62 years). A rate of 9.38% of our population was under 25 years old. For the 59 M-sex patients, 47.83% presented themselves to explore an infertility problem. Of the 37 women, 48.53% complained of leucorrhea. The majority of the 96 patients (78.13%, n = 75) said they had never had a medical history. Most of those with a history of sexually transmitted infection (STI) did not know exactly which germ was responsible for the infection, or whether several agents had been diagnosed. The vast majority (n = 90, 93.75%) did not have a surgical history; and when they are reported, they are urological or gynecological-obstetrical. Of the 96 patients, 8.33% (8/96)

reported taking an antibiotic. But the antibiotics in question were all in the beta-lactam family and did not represent an exclusion criterion. A total of 27 patients (28.13%) claimed to have engaged in sexual vagrancy (multiplicity of partners) during a period of their life, more or less old, that we could not date accurately.

The overall prevalence of Uu infection was 32.29%. In men, it was 28.81% against 37.84% for women. The overall prevalence of MH infection was 9.38%. It reached only 5.08% in men and was, on the other hand, high in women 16.22%. Co-infection at Mh + Uu showed an overall prevalence of 5.21%. The statistical analysis was made without distinction between the Uu or Mh infection by retaining as variable the positivity or not of the test with respect to the MUG infection. All the results showed the absence of statistical linkage of the MUG infection with the parameters studied (p >> 0.05) (Table 1). The resistance of Uu was close to 10% against doxycycline and azithromycin vs 0% and 83% for Mh, and none against levofloxacin for both germs.

		Patients	MUG+	MUG-	Prévalence	p. Value
Terrain	Sexual vagabond	27	11	16	40,74%	0.892
Type of Sampling	1er jet d'urine	3	0	3	0,00%	0.515
	Urethral sampling	7	1	6	14,29%	
	Vaginal sampling	34	14	20	40,00%	
	Vulvar collection	2	1	1	50,00%	
	Sperm	50	19	31	38,00%	
Sex	Fémale	37	15	22	40,54%	0.659
	Male	59	20	39	33,90%	
Women's Reasons for Consultation	Leucorrhées	33	14	19	42,42%	0.654
	pruritus	17	10	7	58,82%	
	Pregnancy	2	1	1	50,00%	
	Yellowish discharge	1	0	1	0,00%	
	metrorrhagia	1	0	1	0,00%	
	Burning micturition	9	5	4	55,56%	
	Low pain	5	1	4	20,00%	
Reasons for consultation man	Burning micturition	18	3	15	16,67%	0.519
	Pains of the testicles	7	4	3	57,14%	
	Urethral discharge	14	3	11	21,43%	
	subfertility	2	1	1	50,00%	
	Infertility	44	15	29	34,09%	
	Penis pain	3	1	2	33,33%	
	Hemospermia	1	0	1	0,00%	
	Pruritus	2	0	2	0,00%	
	Pruritus in the spouse	1	0	1	0,00%	

Table 1: Prevalence of MUG infection by study variables.

Discussion

The diagnosis of colonization or MUG infections uses different kits for the detection and quantification of Uu and Mh in broth adapted from genital specimens [5]. Example: IST2 BioMerieux, MycoView from Zeakon. These systems generally correspond to single microplates (one per patient). Some galleries allow to determine at the same time the antibiotic susceptibility of the strain of mycoplasma detected [6], the antibiotics to be tested being in single or in several concentrations. However, there are problems of interpretation making their quantification necessary because of their presence in the commensal state (changing unit staining or UCC) [3]. Molecular biology techniques such as Polymerase Chain Reaction (PCR) are of no interest in the routine diagnosis of Uu and

Mh at the urogenital level unless it considers quantification or if it is applied to samples from normally sterile anatomical sites whose samples do not lend themselves well to culture such as fluid or joint biopsies. But they remain the only methods applicable for the diagnosis of Mg. On the contrary, the serologies are not adapted to the diagnosis of MUG infections.

Few Moroccan studies have addressed the prevalence of MUGs. However, our results in the prevalence of Uu, Mh and Mh + Uu are higher than those reported by Bouzaidi R [7] and Mohammadi F [8]. On the other hand, they are comparable to those of an old study [9] carried out at the Institut Pasteur on 1640 patients who presented themselves to the care of the "MST" clinic of this insti-

tute, despite its not specified the prevalence of Uu and Mh separately. This is in line with the most likely nature of our study population and is likely similar to this one (Table 2).

Référence	Population	Prevalence of Uu (%)	Prevalence of Mh (%)	Prévalence of Uu + Mh (%)
Heikel J ; 1999 [9]		Prévalence des MUG		
	H : PSMST	25.94*		
	F : PSMST	45.38*		
Thèse Ph n° 96 2011 [8]	H: symptomatic	17	2.8	/
	F: symptomatic	20	2.3	/
Thèse Ph n° 75 2013 [7]	H: symptomatic	8.33	2.7	2.7
	F: symptomatic	4	0.5	0.5
Notre étude	H: symptomatic	28.81	5.08	5.21
	F: symptomatic	37.84	16.22	

Table 2: Prevalence of MUG reported in Morocco.

*Percentages for which there is no statistically significant difference with those in our study.

PSMST: Patients Specialized Services in Sexually Transmitted Diseases.

Our results concerning MUG, in relation to international data are given in the table below (Table 3). They are different from those of Western countries, but quite similar to those noted in Tunisia, Brazil or China, countries with a great socio-economic and cultural similarity with Morocco.

In Poland, Bałajewicz-Nowak M., *et al.* [10], conducted a study to evaluate the frequency of cervical Uu and Mh infections in women who had normal pregnancies and others who had symptoms such as abortions or premature deliveries. A total of 73 endocervical samples were investigated for Uu and Mh using the BioMérieux Mycoplasma IST2® Test. Mh was present in 6.8% and Uu in 28.8% of samples. These frequencies are similar to those of our study.

In addition, MUG infections, in addition to other bacterial STI agents, are a major risk factor for Human Papilloma Virus (HPV) infection, the oncogene effect of which is well known. Thus, Shigehara K., *et al.* [11] analyzed the risk factors for HPV infection in the urethra by determining the prevalence of various microorganisms: *Neisseria gonorrhoea* (Ng), *Chlamydia trachomatis* (Ct), Mg, Mh, Uu, Up, Gv and HPV in 176 male Japanese patients with urethritis and removed by brushing the distal urethra. The agents sought were detected at respective levels of (in%): 19 - 26 - 18 - 12 - 12 - 8.5 - 14 and 20. The multivariate analysis indicated that more than 4 sexual partners in the year previous and the presence of Ng and/or Ct and/or Mg were independent risk factors for HPV urethral infection. In our study, no patient of either sex was explored for HPV; and this information deserves to be reported, but it also requires other laboratory techniques that we do not have now.

Référence	Sampling and methods	Population	Uu (%)	Mh (%)	Mh+Uu (%)
Ito S 2012 Japan [17]	PJU PCR	Men < 40a with and without epididymitis	10,3*	6*	/
Al-Sweih NA 2012 Kuwait [18]	Sperm According to WHO	Men			
		Infertile	26,1	32,4	8.7
		Fertile	24,4	17,1	11.7
Zhou Y 2011 China [24]	Cervical ?	Women with tubal infertility (st)			
			23,24	38,23	29.05
Liu J 2014 China [25]	Semen according to WHO PCR Culture on specific medium	Infertile men	26.57*	5.98*	5*
		Controls	24.88*	4.88*	4.39*
Aydin Y 2010 Turkey [26]	Liq. Périton.	F speakers	26	Basse	/
	Cervical PCR	Controls	15,3	Basse	/
Rodrigues 2011 Brazil [27]	Endocervical PCR	Women consultants	38,4*	21,9*	/
Gdoura R 2008 Tunisia [28]	PJU, sperme PCR Hybrid maison	Infertile men	18,3*	9.6*	/
Ghosh A 2011 India [29]	PJU MUG : culture	HIV + naive for trt and control	6	1	0
			2	0	0
Mc Iver CJ 2009 Australia [30]	Endocol	FSA (SAW)	Up 57	13,7	1,3
	PCRmx	Control	Uu 6,1		
Günyeli I 2011 Turquie [31]	Urethral Endocervical ELISA	F infertile	20.8	1.9	/
		F controls	41.5	3.8	/
		H infertile	24.5	3.8	/
		H controls	37.7	7.5	/

Balajewicz 2011 Poland [10]	Endocol MUG : IST2	Women abortion or premature birth	28,8	6,8	/
Notre étude 2014	PJU, PU, Endocol, Sperme ICG Mycoplasma Test	Infertility and other motives	32,3	9,4	5.21
		H	28,8	5,1	
		F	37,8	16,2	

Table 3: Prevalence of MUG reported in different countries.

*Percentages for which there is no statistically significant difference with those of our study by the comparison test.

These sometimes-important differences between Uu and Mh prevalence's are a function of the techniques and samples used, the populations studied and the countries, but all show that MUGs are more frequent in subjects at risk and/or presenting a patent urogenital infection; but sometimes statically insignificant, which reinforces screening recommendations in populations with risk factors through clinical examination and history. It should be noted that self-medication [12] and non-generalization of molecular biology contribute to the underestimation of the true incidence of these infections in Morocco, especially those related to Mg.

In this study we tried to identify risk factors possibly associated with MUG infection using multivariate analysis. No predisposing factor statistically associated with MUG infection was found ($p > 0.05$). The absence of any statistical association between their presence at high levels demonstrated by the UCC/ml counting cup to be greater than 10×10^4 /ml; signing an infection; and a possible risk factor or demographic characteristic is probably due to the fact that MUGS are commensals of the urogenital flora widely distributed in the population. Nevertheless, we noted that their frequencies vary considerably, firstly according to the age (the frequency increases after puberty and decreases when the sexual activity decreases), and secondly according to the sex (more important for the woman) and in last place depending on the pregnancy, which proves to be

a favorable factor. All these findings revealed by our study, are in agreement with published data including a Polish study that aimed to determine the presence of MUGs in the cervical canal of the uterus in 222 women and whose results did not show any evidence of association between the presence of MUG and any epidemiological determinant [13]. Although, the multiplicity of sexual partners, the low socioeconomic level, oral contraception and the history of STIs have been identified as risk factors for infection by several authors including Pinna and Ruzman [14,15].

According to the European recommendations, the first-line treatment of uncomplicated urogenital infections, such as the infections that are the subject of this work, uses azithromycin at a dose of 1 g per os, in a single dose, or at doxycycline 100 mg per os, twice a day, for seven days. Therapeutic alternatives are based on erythromycin base (500 mg, 4 times/day for 7 days), ofloxacin (300 mg twice daily for 7 days) or better, levofloxacin (500 mg once daily) for 7 days [16]. The most interesting molecules are azithromycin, doxycycline and levofloxacin, as demonstrated by our study and many other studies by low levels of resistance (Table 4). In all cases, the treatment of partners is mandatory, as is the need to prolong the duration of treatment for deep infections and the interest of associating other antibiotics with other microorganisms that may be involved, particularly in salpingitis.

Références	MUG and staff	% R											
		MIN	DOX	ERY	AZI	JOS	THI	CLI	CLA	ROX	OFLX	SPA	LEV
Leli C 2012	Mh (6)	/	0*	100*	66.7*	0*	/	/	/	66.7*	0	/	/
Italie [1]	Uu (152)	/	0	0	0	0*	/	/	/	0	27.6	/	/
Bayraktar M 2010	Mh (5)	/	0*	100*	40*	0*	/	/	40*	/	60	/	/
Turquie [20]	Uu (27)	/	0*	22.2*	22.2*	0*	/	/	7.4*	/	85.2	/	/
Diaz L 2013	Mh (50)	10*	18*	94*	92*	/	/	14*	86*	/	70	/	/
Cuba [21]	Uu (154)	16.2	16.9*	46.1	30.5	/	/	18.2	63	/	64.3	/	/
Zhu C 2012	Mh (50)	1.75	1.75*	/	85.9*	5.26*	/	/	86*	89.5*	47.37	42.1*	35.1*
Chine [22]	Uu (1538)	1.5	1.56	/	15.2*	11.96	/	/	6.05*	33*	32.96	27.37	20.09
Kechagia N 2010	Mh (5)	/	0*	80*	80*	0*	/	/	80*	/	20	/	/
Grèce [23]	Uu (111)	/	0	33.3	8.1*	0*	/	/	6.3*	/	18.1	/	/
Th. Pharm. N°75 2013 Maroc [7]	Mh(5)	/	0*	60*	80*	0*	/	/	60*	/	80	/	/
	Uu (30)	/	3.3*	6.67*	0*	0*	/	/	6.67*	/	66.67	/	/
Notre étude 2014	Mh (6)	16.7	0	83.3	83.3	0	50	33.3	66.67	83.33	/	83.3	0
	Uu (32)	9.38	9.38	12.5	9.38	0	25	62.5	12.5	18.75	/	0	0

Table 4: Resistance profiles of Mycoplasma hominis and Ureaplasma urealyticum reported in different countries.

* Percentages for which there is no statistically significant difference with those of our study by the comparison test.

In 2005 and 2004, Turkish authors had, respectively, used the ELISA test and the IFD and IST2 methods to study, on the one hand, the effect of azithromycin monodose versus doxycycline, on the other hand to study the prevalence and the efficacy of Ct, Uu and Mh treatment in patients with non-gonococcal urethritis (UNG) on urethral and endocervical specimens and demonstrated the superiority of azithromycin [17,18]. Luo DQ., *et al.* revealed in a study that tetracycline possessed low MICs against Ureaplasma when used as monotherapy [19]. In this same work, the combined treatment was demonstrated to be effective by an in vitro study of combinations of dual therapy erythromycin + tetracycline and levofloxacin + tetracycline showing significantly reduced MICs, unlike the combination of erythromycin + levofloxacin. They recommend using one of the two combinations for greater therapeutic efficacy.

The results of our study are listed in table 4 and are broadly consistent with those of other authors around the world. It can be noted that our results are similar to those of Leli C., *et al.* [1], Bayraktar M., *et al.* [20], Diaz L., *et al.* [21], Zhu C., *et al.* [22], Kechagia N., *et al.* [23], doctoral thesis in pharmacy, Rabat, Morocco 2013 [7]; for doxycycline, erythromycin, azithromycin, josamycin, roxithromycin and clarithromycin. They also join the results of Zhu C., *et al.* [22] for sparfloxacin and levofloxacin. In contrast, minocycline, reported by two authors, is consistent with our results only in one case. The table also highlights the existence of some divergence of results especially when it comes to Uu. Chinese and Turkish studies show maximum agreement for both Uu and Mh [20,22]. There are molecules that are impossible to discuss, those that are not tested in the gallery we used (tetracycline, pristinamycin, ciprofloxacin, ofloxacin) as well as those that the other authors have not tested at all and which are on our list (thiamphenicol, gatifloxacin). These molecules do not therefore constitute discrepancies with the listed studies. To consolidate the benefit of *Mycoplasma* kits like the one we used for both diagnosis and treatment and follow-up help, the work of Zhou Y., *et al.* [24] in China is an example as it explored the effects of MUGs and Ct on tubal infertility and assessed the sensitivity and resistance of isolated MUGS from female TUG to guide the rational use of antibiotics. A squad of 327 women with tubal infertility vs 286 unscathed was explored.

The susceptibilities of Uu were: roxithromycin 96.05%; josamycin 96.05%; tetracycline 82.89%; doxycycline 92.11%; clarithromycin 96.05%; therefore, relatively high but lower vis-a-vis ciprofloxacin and spiramycin. The susceptibilities of Mh were: josamycin 95.83%; doxycycline 91.67%; minocycline 83.33% and actinospectacin 75.00%; therefore, relatively high but lower compared to erythromycin, azithromycin, roxithromycin and clarithromycin. The Uu + Mh combination was highly sensitive to josamycin (90.52%), high resistance (77.89% - 91.58%) to erythromycin, clarithromycin, azithromycin, spiramycin, ciprofloxacin and ofloxacin. Thus, Uu or Mh infection in women with tubal infertility has a higher frequency than in the fertile population. For many key antibiotics in the treatment of MUG infections, the Uu + Mh combination exhibits strong resistance because additive type "A" union "B" sets; This fact must be taken into account in the diagnosis and treatment.

Conclusion

Our results are consistent with data from the national and international literature on both the high prevalence of Uu compared to Mh and the resistance profiles of these two antibiotic germs. The classical methods of diagnosis such as that used in our study are of acceptable performance with one exception, because they are not adapted to Mg, very tedious, which requires molecular bi-

ology techniques. The extent of infections caused by this species is therefore unknown. The association Uu + Mh complicates the therapeutic management and emphasizes the interest of the kits of this kind. Solutions must be made regarding the pre-analytical phase to avoid any negative influence on the results. In addition, the molecular diagnosis must be part of our technical platform to first separate Uu from Up and specially to know the prevalence Mg.

Conflicts of Interest

The authors do not declare any conflict of interest

Contribution of the Authors

All the aforementioned authors contributed to the realization of this article from the collection of data until the final drafting of the work.

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