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Urogenital Mycoplasma in Moroccan Population: Prevalence and Antibiotic Susceptibility

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ABSTRACT

Mycoplasmas are the smallest identified organisms found in plants, animals and humans, some of their species are associated with severe uro-genital diseases in women and men, causing various health problems. The aim of our study is to evaluate the rate of urogenital Mycoplasma infection in Moroccan population, and study the circulating strains sensitivity to antibiotics.

Our study focused on 2 populations: one with controlled sexual behaviors (population 1), and the other with risky sexual behaviors (population 2). The method used was culture in liquid medium using the Mycoview kits "Zeakon diagnostics France"; the method is based on specific metabolic properties of the Mycoplasma species.

The results obtained showed that urogenital mycoplasmas prevalence was considerable, and depends of the studied groups. Indeed, 36% males and 21% females were reveled positives in population 1, against 41% males and 59% females in population 2. Regarding mycoplasma species prevalence, we found a distinct disproportion in the incidence of the two mycoplasmas. *U. urealyticum* was more commonly detected than *M. hominis* in all studied groups. The analysis of the resistance to antibiotic profiles according to the gender showed high level of resistance especially to azitromycin and roxymicin in both of two populations. The comparison of resistance profiles in both populations showed also that strains detected in patients belonging to the controlled sexual behaviors population were found more resistant. The comparison of the antibiotic resistance profiles according to gender showed that strains detected in females present high level of resistance to all used antibiotics. However, strains detected in male patients present lower resistance to antibiotics, and no resistance was observed to josamycin and minocycline in males.

This investigation allowed to get a first idea on urogenital mycoplasma prevalence and antibiotic resistance in Morocco, to conclude that strain sensitivity to antibiotics depends on both gender and sexual behavior; and seems to be related to the frequency of antibiotic use. To control mycoplasma infection, we recommend performing antimicrobial tests before any medical prescription, and not resorting to self-medication.

Keywords: *Ureaplasma urealyticum, Mycoplasma hominis, prevalence, antibiotics susceptibility.*

1. INTRODUCTION

Mycoplasmas are the smallest identified organisms found in plants, animals and humans (Tully et al., 1981; Yoshida et al., 2002) These bacterial agents are found in the saprophyte state in humans; however some of these species are associated with severe urogenital diseases in women, men and children (Cassell et al., 1991), causing various health problems. The urogenital mycoplasmas are recognized as responsible for sexually transmitted diseases, and implicated in major public health problems. These microorganisms possess the smallest genomes (Fraser et al., 1995), among self-replicating free-living organisms. They can be considered as minimal bacterial cell prototypes, with reduced metabolic abilities. Interestingly, in addition to their distinct pathogenic roles, they also have different energy-generating pathways. *M. genitalium* is a glycolytic species, whereas *M. hominis* and *Ureaplasma spp* are both nonglycolytic species, producing energy through arginine degradation or urea hydrolysis respectively. Nongonococcal urethritis (NGU) caused by *Chlamydia trachomatis* and mycoplasma species are common cause of symptomatic and asymptomatic infections in both of men and women in developing countries (Bordy 1998; Bebear et al., 1997), but there are only few studies about their prevalence and pathogenesis in Morocco. The study of Benchekroun *et al* in 1996 reported 40% urogenital mycoplasma positive cases in STD clinic consultants (Benchekroun et al., 1996).

The aim of our study is to evaluate the urogenital mycoplasma (*Mycoplasma hominis* and *Ureaplasma urealyticum*) infection rate in Moroccan population, and study the circulating strains susceptibility to antibiotics. Furthermore we determine the statistical significance of sexual behavior on the occurrence of infections and other health issues.

2. MATERIALS AND METHODS

Patients

Two hundred and sixty seven patients consulting at gynecology clinics were send to the Sexually Transmitted Diseases laboratory in Institut Pasteur du Maroc for biological examinations. Prior, written consent was obtained from each patient.

Patients belong to two populations: p1 (n=128) with controlled sexual behavior, including 36 men (G1) and 92 women (G2) and p2 (n=139) represents patients with risky sexual behavior, including 49 men (G3) and 90 women (G4).

A questionnaire was completed for each patient, recording personal data (age, sex, condom use), sexual history and clinical data (urinary symptoms, urine frequency, supra pubic/pelvic pain, vaginal and urethral discharge, fertility) were taken. Endo-cervical swabs from females and urethral swabs from males were collected at the same time and discharged in transport medium. Patients should not have urinated for at least 2 hours and should not have taken any antimicrobial agent prior to sampling.

Methods

Mycoplasma hominis and *Ureaplasma urealyticum* are not routinely detected by conventional bacteriological methods. For this study, we opted for culture in liquid medium using the Mycoview kits "Zeakon diagnostics France". The kit contains strips that give information on the presence or absence of *Ureaplasma urealyticum* and *Mycoplasma hominis*, and also provide additional information on antibiotic susceptibility to azytromycin (4µg/mL), Josamycin (4µg/mL), Roxymicin (4µg/mL), Doxycycline (8µg/mL), Minocycline (8µg/mL), Ofloxacin (4µg/mL) and Nofloxacin (2µg/mL). The method is based on specific metabolic properties of the Mycoplasma species: metabolism of urea by *Ureaplasma urealyticum*, arginine by *Mycoplasma hominis,* and the natural resistance of *Ureaplasma urealyticum* and *Mycoplasma hominis,* respectively to lyncomycin and erythromycin.

Swabs in the transport medium were processed according to the manufacturer's instructions; they were vortexed rapidly and were used to rehydrate the lyophilized growth medium provided in the kit. The Mycoplasma strips are then inoculated with 100 µl of the rehydrated growth medium overlaid with two drops of mineral oil, then incubated at 37°C and observed for color changes. The results were interpreted after 24h and 48h of incubation.

The results were interpreted according to the manufacturer's instructions. Samples with titles $\geq 10^4$ UCC are considered positive.

Statistical analysis was performed using the MS EXCEL and SPSS. Categorical variables were analyzed by chi square (χ^2). *P-values* of <0.05 were considered statistically significant.

3. RESULTS

The study was enrolled on 267 patients, constituted from two populations, the first one, with controlled sexual behaviors patients: P_1 (n=128) including 36 men (G_1) and 92 women (G_2), with a mean age of 36 years, and known for their unrisky sexual practice (intra-conjugal sexual practice). The second population: P_2 (n=139) including 49 men (G_3) and 90 women (G_4), with mean age of 33 years, is composed from patients with risky sexual behaviors.

The clinical data analysis in both populations showed that urethritis is dominant, with (n=23) 64% in controlled sexual behaviors male patients, but in even high proportion (n=48) 97% in risky sexual behaviors. The χ^2 value (17.51) and (p<0.001) suggest that urethritis is related to risky sexual behavior in males (Table 1). In female patients, the vaginal discharge was the most represented symptom, with 27% in controlled sexual behaviors female patients against 45% in risky sexual behaviors (Table 1). The χ^2 value (6.65) and (p<0.01) suggest that vaginal discharge is related to risky sexual behavior in females (Table 1). In contrast, the results showed that there is no influence of sexual behaviors on infection/ailment outbreak for pelvic pains (females), cervicitis (females) and pruritis (male and female) at p>0.05 (Table 1). It is however noticeable that there is difference in pruritis occurrence between male and females having risky sexual behavior p<0.05 (Table 1). For the controlled sexual behavior, It is noticeable that there is no difference in pruritis occurrence between male and females (p>0.05), therefore, there is no influence of gender on pruritis outbreak in controlled sexual behavior (Table 1).

Table 1: Clinical symptoms

		Clinical Symptoms		
		Controlled sexual behaviors, P_1 (n=128) (Male n=36, female=92)	Risky sexual behaviors, P_2 (n=139) (Male n=49, female=90)	*P value*
Male patients	Uretritis	23(64%)	48(97%)	p<0.001
	pruritis	4(11%)	1(2%)	p>0.05
	Infertility	9(25%)	-	
Female patients	Vaginal discharge	25(27%)	41(45%)	p<0.01
	Pelvic pains	22(24%)	17(19%)	p>0.05
	pruritis	9(10%)	14(15%)	p>0.05
	cervicitis	22(24%)	19(21%)	p>0.05
	Mictional burns	7(8%)	-	
	avortement	3(3%)	-	
	Infertility	4(4%)	-	

Urogenital mycoplasma infection (*Ureaplasma urealyticum*, *Mycoplasma hominis*) prevails in patients with controlled sexual behaviors and risky sexual behaviors. It occurred in 39% of male patients against 21% of female patients in controlled sexual behaviors patients and occurred in 41% in male patients against 59% in female patients in risky sexual behaviors (Table 2).

Table 2: Comparison of Mycoplasma prevalence according to the Sexual behaviors

	Male Patients		Female Patients	
	controlled sexual behaviors	risky sexual behaviors	controlled sexual behaviors	risky sexual behaviors
Prevalence	39%	41%	21%	59%
P value	>0.05		<0.0001	

As indicated by the χ^2 value (19.871) and $p<0.0001$, Suggesting that mycoplasma infection is related to risky sexual behavior in females. Indeed, the difference is significant between female Patients of two groups. However, the sexual behavior is insignificant on mycoplasma infections for male patients ($p>0.05$). Furthermore, male patients with controlled sexual behavior are more likely to experience mycoplasma infection than females of same group ($p<0.05$). In contrast, the difference is not significant ($p>0.05$) for mycoplasma infection according to gender in risky sexual behavior groups (Table 3)

Table 3: Comparison of Mycoplasma prevalence according to the gender

	controlled sexual behaviors		risky sexual behaviors	
	Male Patients	Female Patients	Male Patients	Female Patients
Prevalence	39%	21%	41%	59%
P value	<0.05		>0.05	

The prevalence of mycoplasma species was also evaluated separately in males and females in both of two populations. The results showed that *Ureaplasma urealyticum* (UU) is the most prevalent species; it is detected among 39% male patients in controlled sexual behaviors population against 10% of male in risky sexual behaviors population (Table 4). *Mycoplasma hominis* (MH) is detected associated to *Ureaplasma urealyticum* (UU) in 31% of male patients in risky sexual behaviors population (Table 4). In female patients, *Ureaplasma urealyticum* was detected in 16% of controlled sexual behaviors population against 49% in risky sexual behaviors population. In the same groups, *Mycoplasma hominis* was detected associated to *Ureaplasma urealyticum* in 5% among controlled sexual behaviors population and alone in 10% among risky sexual behaviors patients (Table 4).

The results show that the UU prevalence is gender related in both of controlled and risky sexual behavior groups ($p<0.0001$). This means that in the controlled sexual behavior group, males are significant more likely to have UU than females ($p<0.01$), while in the risky sexual behavior group, females are more likely to have UU than males ($p<0.0001$) (Table 4).

Table 4: Urogenital mycoplasma strains prevalence

	Patient groups	Mycoplasma	Positive cases counts	Prevalence	*UU prevalence comparison according to the gender P value*
Controlled sexual behaviors population (n=128)	$Group_1$ (n=36)	UU	14/36	39%	$p<0.01$
		MH	0/36	0%	
		UU+MH	0/36	0%	
	$Group_2$ (n=92)	UU	15/92	16%	
		MH	0/92	0%	
		UU+MH	5/92	5%	
Risky sexual behaviors population (n=139)	$Group_3$ (n=49)	UU	5/49	10%	$p<0.0001$
		MH	0/49	0%	
		UU+MH	15/49	31%	
	$Group_4$ (n=90)	UU	44/90	49%	
		MH	9/90	10%	
		UU+MH	0/90	0%	

The antimicrobial susceptibility of the total identified mycoplasma showed various and heterogeneous responses to the tested antibiotics, the method used can't show the sensitivity of mycoplasma species separately. To study the resistance to antibiotic levels, a comparison of the resistance profiles was carried out according to gender and the sexual behaviors. The comparison of resistance profiles in both of two populations showed that strains detected in controlled sexual behaviors population patients were found more resistant, this resistance is observed to all used antibiotics (Fig 1).

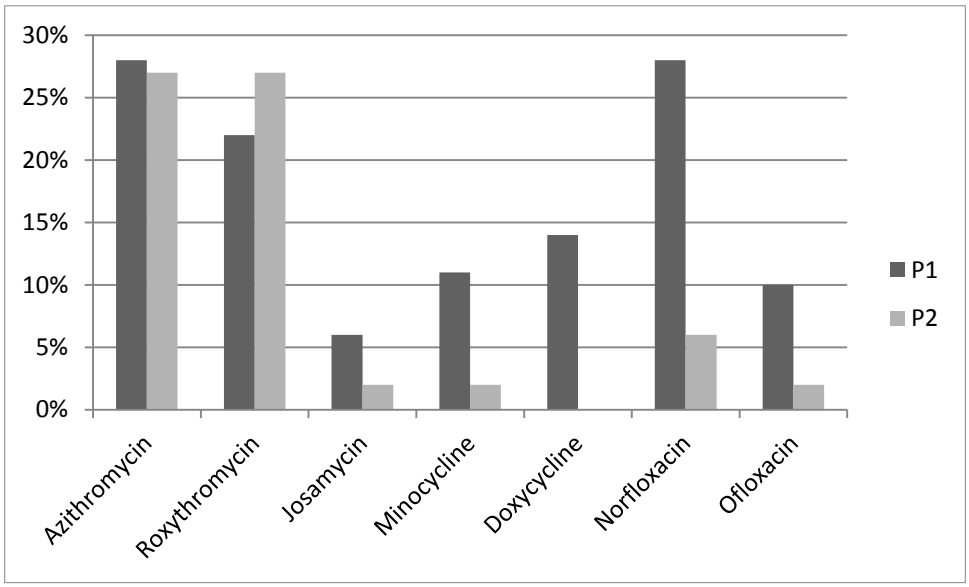

Fig 1: Resistance profiles according to the Sexual behaviors
P1: Controlled sexual behaviors population
P2: Risky sexual behaviors population

The profiles analysis according to the gender showed a high level of resistance especially to azitromycin and roxymicin in strains detected in both of male and female patients. The comparison of the antibiotic susceptibility profiles according to gender showed also that strains detected in females present high level of resistance to all used antibiotics. However, strains detected in male patients present lower resistance to the antibiotics, and no resistance was observed to josamycin and minocycline in strains detected in males (Fig 2).

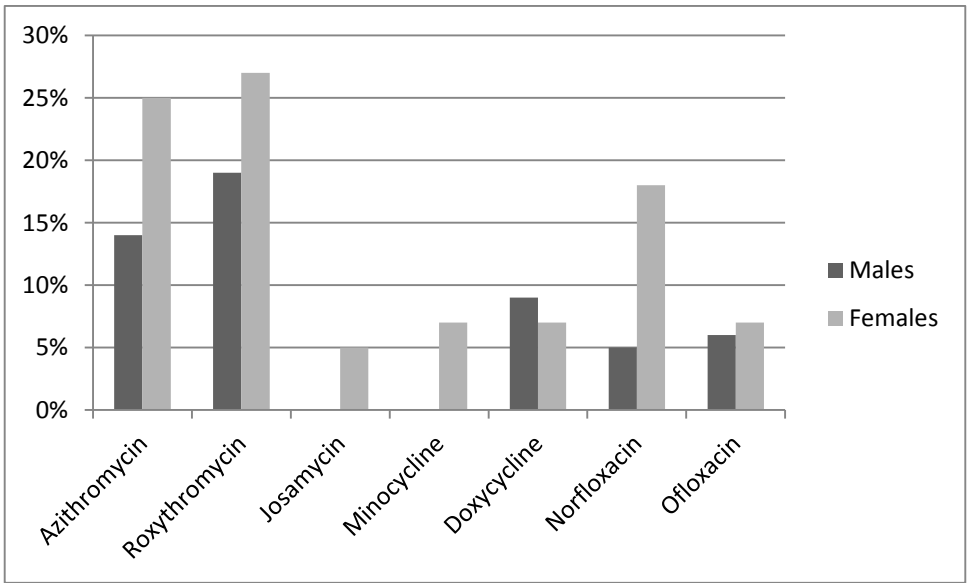

Fig 2: Resistance profiles according to the gender in two populations

4. DISCUSSION

Urogenital mycoplasmas have been shown to cause genital tract diseases in humans. *Ureaplasma urealyticum* is considered as the causative agent of non-gonococcal urethritis (NGU) and was detected in 10% to 40% of the cases. Untreated, urethritis may be complicated by epididymitis or prostatitis and may play a role in urinary calculi formation in patients with abnormalities of the urinary tract. Mycoplasmas are also responsible for sub-fertility and were frequently isolated during the couple's infertility testing (Tardieu et al., 1988; Gdoura et al., 2007). In women, infections may be asymptomatic and colonize the genital tract causing cervicitis or salpingitis.

In our study, we investigated the urogenital mycoplasma infection and the strains susceptibility to antibiotics; we opted for culture in liquid medium method, using Mycoview kits (Zeakan diagnosis, France).

The clinical data analysis revealed that urethritis was the dominant symptom in males of both two populations with 64% in controlled sexual behaviors population against 97% in risky sexual behaviors. This in average higher than the 63% cases of urethritis reported by Schlicht et al among sexually active young adults male patients (Schlicht et al., 2004).

In women of both studied populations, clinical data were highly heterogeneous. Indeed, we found vaginal discharge, cervicitis, pelvic pain and pruritus. The vaginal discharge was the most represented symptom in female patients, with 27% in controlled sexual behaviors against 45% in risky sexual behaviors. Schlicht et al. also reported that vaginal discharge was the most represented symptom in his female patients, it was observed in 86% patients (Schlicht et al., 2004).

The two species of urogenital mycoplasma (*Ureaplasma urealyticum*, *Mycoplasma hominis*) were detected in 39% in male patients against 21% in female patients of controlled sexual behaviors population, and 41% in male patients against 59% in female patients of risky sexual behaviors population. Both of males and females are more affected in risky sexual behaviors. Schlicht et al, focusing their study on symptomatic sexually active male and female adult patients detected mycoplasmas in 62% males and 54% females (Schlicht et al., 2004). Statistical analysis in our results suggests that mycoplasma infection is related to risky sexual behavior in females, indeed, the difference is significant between female patients of two studied populations, similar than our results, numerous studies related Urogenital mycoplasma infection to risky sexual behaviors (Willcox 1975, Schlicht et al 2004. Baykartar et al 2009).

The prevalence of mycoplasmas separately was also evaluated in both of two populations. The results showed that *Ureaplasma urealyticum* prevalence is higher than *Mycoplasma hominis* prevalence; it was detected among 39% male and 16% female in controlled sexual behaviors patients, also in10% male and 49% female in risky sexual behaviors patients. While *Mycoplasma hominis* was detected associated to *Ureaplasma urealyticum* in 31% male in risky sexual behaviors population and 5% female in controlled sexual behaviors population, it was also detected alone in 10% female in risky sexual behaviors population. Our results are similar to those reported by Sahoo et al in 2000 in their investigation among female consultants; they found 43% positive cases by *Ureaplasma urealyticum* (Sahoo et al., 2000). Similar results are also reported (Bayraktar et al., 2010, Zdrodowska et al., 2006). However, mycoplasma rates detection in our study are lower than those reported by Leng study, where *Ureaplasma urealyticum* was isolated in 60% and *Mycoplasma hominis* in 20% genital infection consultants (Leng et al.,1987), and Di Bartolomeo study conducted in healthy women, where a similar prevalence of *U. urealyticum* was reported at 61.4% (Di Bartolomeo et al., 2002).

In comparison to many previous studies, which have reported between 10% and 70% of women to be colonized with *U. urealyticum* but colonization rate with *M. hominis* less than 30% (Schlicht et al., 2004; Domingues et al., 2003; Keane et al., 2000, Grattard et al.,1995), we found a distinct disproportion in the incidence of these tow mycoplasmas. Indeed, *U. urealyticum* was more commonly detected than *M. hominis* in all the studied groups, these results are fairly consistent with those conducted by Schlicht et al and Bayraktar et al (Schlicht et al., 2004; Bayraktar et al., 2010). It seems that *Mycoplasma hominis* catalyzes the pathogenicity of *Ureaplasma urealyticum.*

Our results also showed that the prevalence in the different groups is important, men are more affected in the controlled sexual behaviors population and women are more affected in the risky sexual behaviors population. The subjects' sexual practice in risky sexual behaviors populations exposes them more to the infection by uro-genital mycoplasmas, particularly the concept of multiple partners (extramarital practice) and unprotected sexual practice (without condoms). In contrast males in controlled sexual behaviors have more commonly these infections than females; these results suggest doubt in sexual behavior of these patients.

The urogenital mycoplasma infection treatment is usually based on an empirical antibiotic therapy using cyclines or macrolides (Taylor et Bebear , 1997; Biernat et al., 2007). However, the emergence of resistant strains is increasing since 1970, when ureaplasma strain resistance to tetracycline was estimated at 10%, and that of *Mycoplasma hominis* at 35% (Taylor et Bebear , 1997). The method we used did not allow studying the resistance of *ureaplasma urealyticum* and *mycoplasma hominis* separately. Our results revealed a large heterogeneity in strains susceptibility to the used antibiotics. Indeed, we found a high level (more than 25%) of resistance to azithromycin and roxythromycin (macrolides) in male and female patients of both populations compared to the same level of resistance that was also observed to norfloxacin in patients of controlled sexual behaviors population. A moderate level of resistance was also observed to doxycycline, minocycline and ofloxacin. The results are in concordance with a study done by Biernat Sudolska-M et al in 2007 and Zuo CX et al in 2006. The comparison of resistance according to gender showed that resistance to azithromycin, roxythromycin and norfloxacin was more observed in female patients, and resistance to jozamycin and minocycline was observed only in female patients.

The resistance profiles exhibit similarity with results found by Krausse R et al and De Francesco M.A et al (Krausse 2010; De Francesco et al.,2013). Zuo CX *et al* made a suggestion in their 2006 study that resistance to antibiotics may increase the pathogenicity of these organisms and more resistance can be observed when two species of mycoplasma occur together (Zuo et al., 2006).

This study allowed us to get a first idea on urogenital mycoplasma prevalence and resistance to antibiotics, to conclude that strain sensitivity to antibiotics depends on gender and sexual behavior; it seems to be related to the frequency of antibiotic use. For good control of mycoplasma infection and therapeutic information, we recommend to perform an antimicrobial test before any medical prescription, and recommend to patients to not resort to self-medication.

Finally, our results encourage us to stress the importance of more mycoplasma infection studies: diagnosis and the follow up of infected subjects, therapy and education.

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