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Prevalence and antimicrobial susceptibility profile of *Mycoplasma hominis* and *Ureaplasma urealyticum* in female population, Gabon

Mohamed Ag Baraïka[1], Richard Onanga[2]*, Berthold Bivigou-Mboumba[3], Arsène Mabika-Mabika[4], Ulrick Jolhy Bisvigou[5], Fousseyni S. Touré Ndouo[6], N. Coumba Touré Kane[7]

[1]Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako, Mali.
[2]Medical Research and Analysis Unit, Bacteriology Unit-HSB, CIRMF, Franceville, Gabon.
[3]Mixed HIV Research Unit and Associated Infectious Diseases, CIRMF, Gabon.
[4]Bacteriology Unit-HSB, CIRMF, Franceville, Gabon.
[5]Research Unit on the Epidemiology of Chronic Diseases and Environmental Health UREMCSE University of Health Sciences-Libreville, Gabon.
[6]URAM Medical Research and Analysis Unit, Bacteriology Unit-HSB, CIRMF, Franceville, Gabon.
[7]CHNU Dalal Jamm Guediaway, eIRESSEF DiamniadioUCAD, Dakar, Senegal.

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ABSTRACT

Mycoplasma hominis and *Ureaplasma urealyticum* are microorganisms involved in many pathologies and intrauterine infections. Vaginal wall swabs were taken from 278 women for mycoplasma detection and to study the antimicrobial susceptibility using the Mycoplasma IST2 Kit (bioMérieux). We were able to document in 278 women enrolled in the study, 64.7% have infections caused by *U. urealyticum,* 22.7% by *M. hominis,* and 19.4 % of coinfections involving both germs. Such infections were observed in age groups between 18 and 42 years. Other socio-demographic characteristics studies have been conducted, which allowed to document that single women were the majority population with 68.7% against 16.5% who were living in couple. In response to our questionnaire, 131 single women enrolled in the study never protected themselves during sexual intercourse compared with 52.9% who reported using at least condom. A total of 260 (93.5%) of women reported having only one partner versus 6.5% having more. The study of strains susceptibility to antibiotics revealed a resistance of approximately 80% for macrolides and 100% for fluoroquinolones. All our isolated strains had a sensitivity of about 90% for Pristinamycin and 70% for Josamycin. This study shows that carriage of mycoplasma infection is high in female population in Franceville and vicinities, what should be an element of cautious use of antibiotic during probabilistic therapy treatment of mycoplasma.

1. INTRODUCTION

Mycoplasma hominis and *Ureaplasma urealyticum* are microorganisms involved in many pathologies and intrauterine infections. They are generally associated with pyelonephritis, chorioamnionitis, pelvic inflammatory disease, endometritis, and other important complications such as prematurity, low-birth weight, spontaneous abortion, and infertility issues [1-5]. Pathologies due to such bacteria are often undiagnosed because of the non-specificity and poverty of their clinical presentations.

Such non-specificity problem has been increased ever since HIV pandemic emergence, which has complicated the germs diagnosis [6]. Mycoplasmas are also bacterial agents, of which several species are found in commensal state in humans they are responsible for respiratory, genital, and systemic infections in immunosuppressed individuals [7,8]. Urogenital colonization varies in the world between 20 and 30% for *M. hominis* and 60–80% for *U. urealyticum* [9]. Treatment of urogenital infections due to mycoplasmas is mainly based on cyclins (tetracyclines), macrolides, and quinolones [10].

In the number of quinolones, fluoroquinolones are attractive choices for treating genitourinary tract infections [11]. Biological data obtained in CIRMF Public Health Laboratory on ambulatory patients show a spread of *Mycoplasma* and *Ureaplasma spp.* infections in recent years, what is, furthermore, associated with antibiotic resistance. As a result, a better knowledge of the epidemiology of resistance to such antibiotics will, on the one hand, improve the therapeutic management of patients while reducing broad-spectrum antibiotics prescription and reduce the impact of such infection in the population, on the other hand. No study to date has considered looking into a comprehensive epidemiology and evaluation of mycoplasma resistance to antimicrobials in Gabon. The purpose of our study was on determining *M. hominis and U. urealyticum* strains prevalence in asymptomatic and assessing the resistance of such strains to some antibiotics.

*Corresponding Author:
Richard Onanga,
Medical Research and Analysis Unit, Bacteriology Unit-HSB, CIRMF, Franceville, Gabon.
E-mail: onangar@yahoo.com

2. MATERIALS AND METHODS

The study was conducted in Franceville at the International Center for Medical Research, (CIRMF). It was a cross-cutting and descriptive study, which was conducted over a period of 4 months on women living in Franceville and vicinities, which have been checked either during gynecological consultation or voluntarily come for mycoplasma bacteriological screening at the medical analysis laboratory of CIRMF. To be included in the study, women had to be at least 13 years old and sexually active. Their consent was to be sought before enrolling.

On inclusion in the study, a detailed medical history and clinical examination were performed for each woman. Such information was recorded on a patient card aimed at collecting first-hand socio-demographic, gestational, and behavioral data. Sampling was performed with a swab after a highlighting of cervix using speculum. In that process, leucorrhea losses were picked at endocervix level for the research of mycoplasma. Since such bacteria have high affinity for cells membranes and mucous, it was deemed important to vaginally scratch the mucosa to collect as many cells as possible for diagnosis and antimicrobial susceptibility testing.

The diagnosis was performed using Mycoplasma IST2 Kit (bioMérieux) in consonance with manufacturer's instructions, specifically for urogenital mycoplasmas. This kit allows the culture, identification, indicative blood count, and determination the susceptibility of *U. urealyticum and M. hominis* to antibiotics. Mycoplasma IST2 principle combines a selective culture broth with a 22-well gallery. The broth is suitable for optimal growth of urogenital mycoplasmas.

The result has been interpreted according to the manufacturer's recommendations (positive for identification), yellow to red color (positive for germ number $\geq 10^4$ UFC/sample). The infection threshold for such microorganisms is set to a specific value greater than or equal to 10^4 ufc/ml, what enables to determine a colonization for a turn of $<10^4$ ufc/ml and an infection for a value greater than or equal to 10^4 ufc/ml. Wells were read in 24 and 40 h at 36 ± 2°C and the reading was valid if only the control was positive. As far as antibiotic susceptibility study was concerned, nine antibiotics molecules from Mycoplasma IST2 Kit identification gallery were tested. These molecules are Tetracycline, Doxycycline, Josamycin, Erythromycin, Azithromycin, Clarithromycin, Ofloxacin, Ciprofloxacin, and Pristinamycin. Such molecules belong, respectively, to the antibiotic families of Cyclins, Macrolides, Fluoroquinolones, and Streptogramines.

The data collected on information sheets were recorded in a database (MS Excel® spreadsheet). Data analysis was conducted using SPSS software, version 20. The value of $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Results

For four months, we received 278 women aged 13 and over. This allowed us to isolate strains, 180 *U. urealyticum* 63 strains of *M. hominis* and 54 strains in coinfection all of them in infection situation. The study of socio-demographic, behavioral and gestational characteristics summarized in Table 1 allowed to argue that single women constituted the majority population with 68.7% compared with 16.5% living in couples. The 23–32 age groups were majority population avec 42.4%, followed by the 33–42 age groups, and accounting for 27% of the study population [Table 2]. In response to our questionnaire, 131 (47.1%) single women enrolled in the study never protected themselves during sex intercourses, compared with 147 (52.9%) who reported having used at least one condom. A total of 260 women, or 93.5% stated having one single partner versus 6.5% having more than two partners.

Table 1: Socio-demographic, behavioral and gestational data

Socio-demographic behavioral and gestational data	Female headcount	Percentage
Marital status		
Married	87	31,3
Single	191	68,7
Total	278	100
Use of condoms		
No	131	47,1
Yes	147	52,9
Total	278	100
Number of partners		
One partner	260	93,5
Several partners	18	6,5
Total	278	100
Pregnancies		
No	243	87,4
Yes	35	12,6
Total	278	100

Our study population consisted of 35 pregnant women or 12.6% of the study population. It has been observed that vaginal colonization or commensal portage by such microorganisms changed with age. Prevalence was high in the 18- to 42-year-old age groups with greater portage in the 23–32 year-old modal age group. Such germs were found in commensalism in our study population, respectively, at 7.2% for *U. urealyticum* and 25.5% for *M. hominis* [Table 2]. In our study, population investigations of urogenital mycoplasma infections showed 65% for *U. urealyticum* and 22.7% for *M. hominis*. Coinfection due to such germs (*M. hominis* and *U. urealyticum*) represented 19.4% in our study population; such infection was more involuted in age groups between 18 and 42 years.

M. hominis and/or *U. urealyticum spp.* isolation in infection situations in pregnant women was mostly found in the first and second pregnancy quarter. Infection by behavioral and gestational status is summarized in Table 3. The susceptibility of isolated mycoplasmas to antibiotics is summarized in Table 4. All strains isolated in infection showed relatively low resistance to Tetracycline, Josamycin, and Pristinamycin ranging from 2.8% to 29.6%. We observed 100% resistance, of *U. urealyticum* not only to Ciprofloxacin but also to *M. hominis* with Azithromycin, Clarithromycin, and Erythromycin. Strains found in coinfection showed a 100% resistance to ciprofloxacin, Azithromycin, Clarithromycin, and Erythromycin. In a particular way, all the *U. urealyticum* strains displayed high resistance to all the tested antibiotic molecules.

4. DISCUSSION

The study is the first of its kind to assess in a comprehensive manner the prevalence and antibiotic resistance profile of Mycoplasma strains (*U. urealyticum and M. hominis*) in Franceville, Gabon. The exclusion of women under childbearing age and the free availability of mycoplasma research examinations at the CIRMF Medical Analysis

Table 2: Prevalence of urogenital mycoplasmas by age group

Age group (year)	*Ureaplasma spp*			*M. hominis*		
	Vaginal colonization (Uu<10^4 UFC/ml)	Infection (Uu≥10^4 UFC/ml)	Sterile culture	Vaginal colonization l (Uu<10^4 UFC/ml)	Infection (Uu≥10^4FC/ml)	Sterile culture
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
13–17	1 (5.0)	2 (1.1)	4 (5.1)	1 (1.4)	2 (3,2)	4 (2.8)
18–22	8 (40.0)	37 (20.6)	5 (6.4)	19 (26.8)	18 (28.6)	13 (9.0)
23–32	6 (30.0)	73 (40.6)	39 (50.0)	23 (32.4)	23 (36.5)	72 (50.0)
33–42	4 (20.0)	50 (27.8)	21 (26.9)	20 (28.2)	17 (27.0)	38 (26.4)
43–52	1 (5.0)	18 (10.0)	8 (10.3)	8 (11.3)	3 (4.8)	16 (11.1)
53 et plus	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)	0 (0.0)	1 (0.7)
Total	20 (100)	180 (100)	78 (100)	71 (100)	63 (100)	144 (100)

Mh: Mycoplasma hominis; Uu: Ureaplasma urealyticum; UFC: colony format unit; n: number of population

Table 3: Results of infectious status according to behavioral and gestational data

Socio-demographic data behavioral and gestational	Presence status of germs		
	U. u Infection *n* (%)	*M. h* Infection *n* (%)	Co-Infection *(Uu+Mh)* *n* (%)
Use of condoms			
No	100 (55.5)	36 (57.1)	23 (42.6)
Yes	80 (44.4)	27 (42.8)	31 (57.4)
Total	180 (100)	63 (100)	54 (100)
Number of partners			
One partner	167 (92.8)	59 (93.6)	50 (92.6)
Several partners	13 (7.2)	4 (6.3)	4 (7.4)
Total	180 (100)	63 (100)	54(100)
Pregnancies			
No	161 (89.4)	57 (90.5)	50 (92.6)
Yes	19 (10.5)	6 (9.5)	4 (7.4)
Total	180 (100)	63 (100)	54 (100)

U.u: Ureaplasma urealyticum, M. h: Mycoplasma hominis, n: Number of population

Laboratory allowed us to eliminate certain selection biases. We were able to document in 278 women enrolled in the study, 64.7% of infection caused by *U. urealyticum,* 22.7% by *M. hominis,* and 19.4% coinfection involving both germs (*U. urealyticum and M. hominis*). Infections alike were observed in age groups between 18 and 42 years. Carriage rate was more significant in such age groups. Carriage and infections caused of mycoplasma this kind may to be explained by this population's sexual activity. In China, authors reported that 31.2% infections caused by *U. urealyticum*, 0.7% by *M. hominis,* and 1.9% coinfections due to the two bacterial germs were believed to be in a population of 6051 women over 4-year period [12].

In Serbia in 2010, it has been reported following a study comparing mycoplasma infections that the prevalence in a symptomatic and asymptomatic infections and coinfections rates in pregnant women population was variable [13]. Such difference observed in all the alike studies could be caused not only to the methodology but also to the epidemiological facies of different epidemiological zones.

Our outcomes show that the prevalence of *Mycoplasma* in Franceville is higher than that in Ouagadougou [14] and Bangui among a women population [15]. Such variation between Northern and Southern countries in terms of mycoplasma infection rate could be explained by the fact that the studies performed in northern countries focused on symptomatic populations, whereas the ones we performed focused on all women broadly speaking a broader comprehensive way focused on achieved in rates.

In all such studies infections and coinfections, distribution profile is the same decreasing order, dominated by *U. urealyticum* followed by *M. hominis* and finally coinfection caused to the two germs (*U. urealyticum and M. hominis)* [12,14-15]. The study of behavioral parameters allowed us to document that single women were the majority population. In response to our questionnaire, 131 single women enrolled in the study did not protect themselves during sexual intercourse that had no real influence on mycoplasma infection rates. Few authors having performed that mycoplasma studies have addressed behavioral parameters. In pregnant women, urogenital mycoplasmas often only bear witness to oestroprogestative impregnation in this particular population [16].

Mycoplasmas naturally resist to antibiotics that act on bacterial cell wall. This is because they do not have a wall. However, they are generally susceptible to antibiotics that inhibit bacterial proteins synthesis [11]. Of all the mycoplasma isolates tested, the overall resistance to fluoroquinolones ranged from 95.2% to 100% for Ciprofloxacin and Ofloxacin. For isolates of *U. urealyticum* and *M. hominis,* overall resistance rates for fluoroquinolones were, respectively, 96.8% and 100% for Ciprofloxacin and 95.2% and 97.2% for Ofloxacin. Such results are higher that, those reported in 2011 in a study which looked at determining the susceptibility of *U. urealyticum* and *M. hominis* strains to antibiotics in northeastern Romania. In the course of said study, a resistance was documented to Ciprofloxacin 75% and 53.7%, respectively, for *M. hominis* and *U. urealyticum*, against 30% and 16.13% for Ofloxacin in *M. hominis* and *U. urealyticum* strains [17].

The explanation for the high percentage of mycoplasma resistance to fluoroquinolones from strains isolated from human patients could not only cause to the frequency of such family of molecules prescription by prescribers in infection treatments but also caused to their mild side effects that could cause such molecules. Macrolide mycoplasmas resistance rates ranged from 69.9% to 100% for all test molecules (Erythromycin, Azithromycin, and Clarithromycin).

Studies achieved in New Guinea and Romania reported resistance rates of mycoplasma ranging from 33.33% to 97.8% for Erythromycin, confirming a high level of resistance to macrolides [17,18]. Such

Table 4: The susceptibility of isolated mycoplasmas to antibiotics

Antibiotic families/ Molecules	*Urea plasma urealyticum* strains (*n*=180)				*Mycoplasma hominis* Strains (*n*=63)				Coinfection (*Uu*+*Mh*) (*n*=54)			
	Susceptible		Resistant		Susceptible		Resistant		Susceptible		Resistant	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Fluoroquinolones												
Ofloxacin	5	2,8	175	97,2	3	4,8	60	95,2	1	1,9	53	98,1
Ciprofloxacin	0	--	180	100	2	3,2	61	96,8	0	--	54	100
Cyclins												
Tetracycline	133	73,8	47	26,1	35	55,6	28	44,4	29	53,7	25	46,3
Doxycyclin	160	88,9	20	11,1	55	87,3	8	12,7	46	85,2	8	14,8
Macrolides												
Azithromycin	35	19,4	145	80,6	0	--	63	100	0	--	54	100
Clarithromycin	61	33,9	119	66,9	0	--	63	100	0	--	54	100
Erythromycin	33	18,3	147	81,7	0	--	63	100	0	--	54	100
Josamycin	147	81,7	33	18,3	47	74,6	16	25,4	38	70,4	16	29,6
streptogramins												
Pristinamycine	175	97,2	5	28	59	93,7	4	6,3	50	92,6	4	7,4

Uu: Ureaplasma urealyticum, Mh: Mycoplasma hominis, n: Number of population

variable rates observed between the results of different studies for the same therapeutic molecules against mycoplasmas, could be due not only to the variability of isolates but also to the attitude of public as regard the therapeutic regimens given by the prescribers. However, mycoplasmas expressed a resistance to Josamycin ranging between 18.3% and 25.4%. Such levels are lower than those of the other molecules of the same family of macrolides. Cyclins resistant (Tetracycline and Doxycycline) was variable in the range of 26.1–44.4%. Such resistance rates are higher than those reported by some authors in New Guinea, Senegal, and Serbia [18-20].

Observed differences could be due to the use of molecules of this family since birth in some African populations. Some mycoplasmas acquire genetic resistance to Tetracycline through a Tet (M) mutation [7,21]. It would be interesting to study the prevalence of Tet (M) mutations, but also mutation genes to fluoroquinolones. In contrast to fluoroquinolones, macrolides, and cyclins, our isolates had a very low resistance to Pristinamycin in the range of 2.8–7.4%. This result shows the good activity of such a molecule on mycoplasma and is a significant a therapeutic agent for infections mainly caused by *M. hominis* and *U. urealyticum*. Studies on the identification of antibiotic resistance genes should be considered in Franceville, where the resistance of mycoplasma to certain antibiotic molecules reaches 100%. Such wide variability in antibiotic resistance profile tested has been reported by several authors throughout the world [22-24].

5. CONCLUSION

The results obtained in this study show that carriage of mycoplasma infection or colonization is high in the female population in Franceville. The treatment based on mycoplasmas probabilistic antibiotic therapy should focus on first-line use of Pristinamycin, followed by Cyclins, although somewhere, their effectiveness is probable on such mycoplasmas.

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7. CONFLICTS OF INTEREST

Authors declared that there are no conflicts of interest.

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