

HIV infection and Mycoplasma co-infection: case-control study in a female population of Douala (Cameroon)

Abstract

Objective: To determine the impact of Human Immuno-Deficiency Virus (HIV) on co-infection with *Mycoplasma hominis* and *Ureaplasma urealyticum* in HIV-infected women.

Methodology: A case-control analytical study based on standardized questionnaire interview and cervical sample collection after informed consent obtained among HIV-positive and HIV-negative women from January 2nd, 2017 to June 30th, 2017 received at the laboratory of Laquintinie Hospital in Douala. Samples collected were used for mycoplasma research, quantification, and antibiogram using the mycoplasma IES kits. Socio-demographic, clinical and biological variables of interest were entered and analyzed on Microsoft Office Excel 2013 and Statistical Package for Social Sciences (SPSS) software version 20 and the chi-square correlation test was used with significance at the threshold of $P < 5\%$.

Results: We analysed 136 samples of women aged 18-65 years, among which 96 HIV+(cases) matched with 40 HIV-(controls). The mean age of the case group was 39.08 ± 10.22 years and that of the control group was 33.28 ± 8.68 years. Genital mycoplasmas were found in 58.3% of cases, with a high frequency for *U. urealyticum* (19.8%) against 2.1% for *M. hominis* and the two germs were associated in 32.3% of cases. In the control group, the carrier rate was 62.5%, with a frequency of 17.5% for *Ureaplasma* alone and no carrier for *M. hominis* alone. Co-infection with the two germs in this group was 40%. The majority of HIV+ women had a CD4 count above 200, and no significant association was found between CD4 count and the presence of mycoplasma in these women ($P=0.094$).

Conclusion: Mycoplasma infection is common in HIV+ women. However, there is no significant association between the CD4 count and the presence of these mycoplasmas.

Keywords: CD4, HIV, mycoplasma hominis, ureaplasma urealyticum

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Abbreviations: HIV, human immuno-deficiency virus; SPSS, statistical package for social sciences; STIs, sexually transmitted infections; AIDS, acquired immune deficiency syndrome; DHS, demographic and health survey; MH, *Mycoplasma hominis*; UU, *Ureaplasma urealyticum*

Introduction

Infection with the human immunodeficiency virus (HIV) is still current in all countries of the world, with 37.9 million people living with HIV, 2.3 million new people infected in 2018 and responsible for 770,000 deaths in 2018.¹ The high prevalence of sexually transmitted infections (STIs) and HIV/acquired immune deficiency syndrome (AIDS) are a real public health problem in many developing countries. It represents approximately 10% of the reasons for consultations in the sexually active population. According to the results of the DHS (Demographic and Health Survey) III 2004, women are more exposed, that is 7.9% of women carrying the virus against 2.5% in men.² Most of these STIs are treated and cured if the diagnosis is made. If left untreated, they can have real complications such as infertility, premature rupture of membranes, neonatal infections, hepatitis or cancer of the cervix; some can be prevented by vaccination such as hepatitis B, the papillomavirus. All these STIs, especially ulcers, weaken the mucosa and increase the risk of HIV transmission. Interest has therefore been paid to these STIs, including: *Mycoplasma hominis* (MH) and *Ureaplasma urealyticum* (UU) infections.

Many studies on this co-infection have been carried out in certain countries, notably in Italy with a prevalence of 41% for UU and 11% for MH,³ 7.5% in Burkina Faso,⁴ 19.3% in the Central African Republic,⁵ a prevalence of 20% among women in Cameroon.⁶ These two bacteria are frequently part of the "normal vaginal" flora. This portage is therefore in most cases safe for human health. *Mycoplasma hominis* and *Ureaplasma urealyticum* are more commonly isolated from adults than children, from pregnant women than from non-pregnant women, and may be more at risk in HIV positive people than in HIV negative people. The presence of these germs in HIV-positive women of reproductive age could therefore complicate their already fragile state of health.

The low availability of information on the link between mycoplasma infection and increased risk of pathogenesis in HIV positive subjects motivated us to conduct this study at the Laquintinie hospital in Douala.

Patients, materials and methods

- Type of study:** It was a prospective analytical case-control study
- Period and duration of the study:** Our study took place from January 02nd 2017 to June 30th, 2017. A duration of six months
- Study population, inclusion and exclusion criteria:** Our study population consisted of both positive and negative HIV patients seen at the clinical biology laboratory at Laquintinie Hospital

during this period. Included were those who were consenting after clarified information, while those who were menstruating or under active antibiotic therapy on mycoplasmas were excluded.

d. Sampling: The sampling was consecutive with a minimum size (N) determined at 106 according to the Lorentz formula (N).

$N = P(1-P) / (Z^2 \alpha / \alpha^2) = 7.5\%$ (prevalence of co-infection of *M. hominis* and *U. urealyticum* in HIV positive subjects = 0.075)⁴

Where,

Z α : 1.96

α : relative risk 5% (0.05)

N \approx 106 Subjects for cases; the size of the control population was defined by convenience as 1/3 of the cases

Materials and methods

Data collection

After addressing and explaining to patients the different information on the research topic, the data was obtained from a pre-tested survey sheet and the CD4 count was recorded confidentially in the HIV+patient book.

Variables studied: The variables of interest were socio-demographic (age, marital status, level of education, type of occupation) clinical (number of sexual partners, use of condoms, history of STI) and biological (HIV typing), mycoplasma, CD4 count).

Pre-analytical phase

- i. **Screening of controls:** Screening of controls (HIV-negative group) was systematically done using the Determine Alere HIV-1/2.
- ii. **Sample collection:** After having comfortably received and installed the patient in a gynecological position, we checked the conformity of the sampling equipment (conforming identification). Next, we exposed the cervix using the speculum and collected from the endocervix with a swab.

Analytical phase

Principle of the test: The “MYCOPLASMA IES” kit allows research, semi-quantitative counting, presumptive identification and antibiogram of *M. hominis* and *U. urealyticum* isolated by clinical samples. The kit is a system of 30 wells containing biochemical substrates and dried antibiotics. The principle of this test is to detect the metabolic activity of urogenital mycoplasmas on a selective medium supplemented with antibiotics, to assess resistance or sensitivity to antibiotics.

After culturing the mycoplasmas, the urea can be broken down by the urease, releasing NH₃ in the case of *U. urealyticum*; arginine can be broken down by arginase, also releasing NH₃ in the case of *M. hominis*. The NH₃ released increases the pH of the medium and the result is obtained by observing the change in color. Antibiotic sensitivity is revealed when there is no change in color due to the inhibition of enzyme activity.

Sample preparation

We first brought the reagents to room temperature, then we added the diluent to the dehydrated medium. The swab used for the sample was immersed in the mixture. We carefully pressed the swab against the wall of the vial, so that the material was evenly dispersed in the physiological solution. A suspension was thus obtained.

Inoculation of the system after bringing the galleries to room temperature (18-25°C), we noted the patient identification number and the date of the start of the examination. We then transferred 100 μ l of the sample suspension to each well in the gallery and covered all the wells with a drop of paraffin oil. We finally covered the gallery with a cover provided for this purpose and incubated the gallery at 37.5°C for 24 hours.

Post-analytical phase

The pathogenicity threshold for a urogenital infection is fixed at 105UCC/ml, which makes it possible to define a colonization for a value <104UCC/ml and an infection for a value \geq 104UCC/ml.

Results interpretation

The results are obtained after a change in color. If the color changes from yellow to red, it implies the growth of mycoplasmas and if the color does not change, it can mean negative growth or sensitivity to antibiotics. Rarely, the color may be pink, and, in this case, the incubation time should be extended from 12 to 24 hours. The strain is sensitive when it is inhibited by the two concentrations of the antibiotic, intermediate when it is inhibited by the large concentration and not the small, resistant when it is not inhibited by either of the two concentrations (Figure 1).

- A. Culture and identification are observed in wells no. 1, 2, 5; well no. 5 for the positive control, well no. 1 for identification of *U. urealyticum*, well no. 2 for the identification of *M. hominis*,
- B. The enumeration is observed in wells no.3 (UU \geq 10⁴) for the enumeration of *U. Urealyticum* and well no. 4 (MH \geq 10⁴) for the enumeration of *M. hominis*,
- C. Antibiotic sensitivity tests are observed in wells 6 to 30.

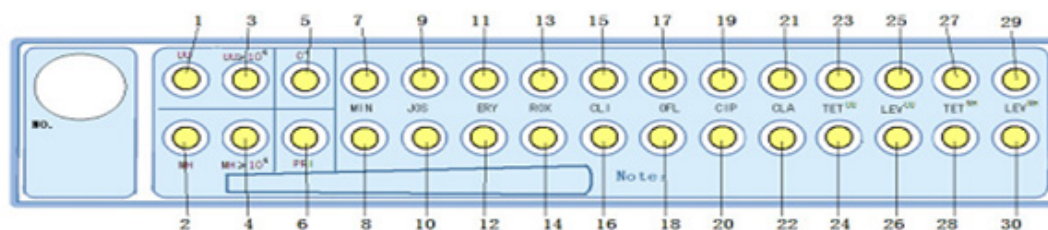


Figure 1 mycoplasma gallery.

Statistical analyzes

Statistical analyzes were performed with Statistical Package for Social Sciences (SSPS) version 20 and Microsoft Office Excel 2013. The chi-square correlation test was used at the significance level of $P < 0.05$

Ethical criteria

We obtained the agreement of the institutional ethics committee of the FMSP (Faculty of Medicine and Pharmaceutical Sciences) of the University of Douala for the realization of this study.

Results

During our study period, we identified 106 HIV positive women constituting the case group, matched to 40 HIV negative women constituting the control group and 10 women were excluded in HIV positive patients (03 not meeting the inclusion criteria and 07 who refused to give consent) Figure 2.

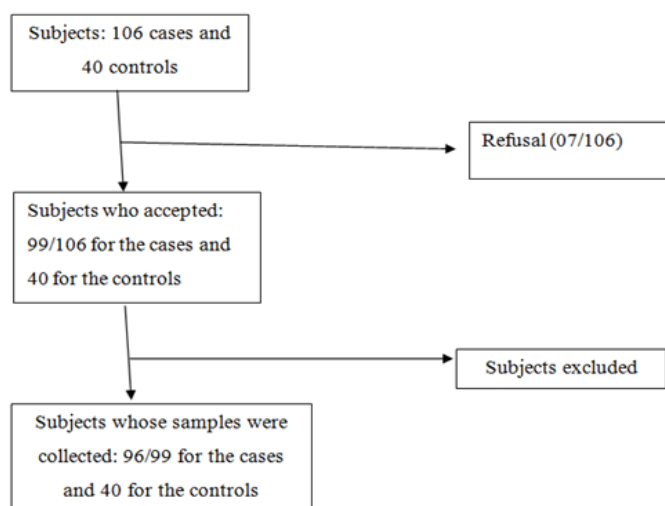


Figure 2 Diagram of participation in the study.

Socio demographic data

The mean age was 39.08 ± 10.22 in cases compared to 33.28 ± 8.68 in controls. The majority age group was that of 38-48 among HIV-positive people: 47% (N=40) and 28-38 among controls: 45% (N=18).

The majority level of study was primary in HIV-positive people: 48.8% (N = 42) and secondary in controls: 52.5% (N=21). Our respondents were mainly single (50% and 65%) and housewives (45.8% and 47.5%) in the two groups with twice as many widows among HIV-positive people than among controls (Table 1).

Clinical data

Type 1 HIV was found in 95.8% of HIV-positive women (N=92) (Table 2).

Most of our respondents declared having only one sexual partner in the two groups with a respective percentage of 60.4% among HIV-positive people (N=58) and 65% (N=26) among controls. On the other hand, 10.4% of HIV+women declared having more than one partner compared to 0% among the controls.

Table 1 Socio-demographic characteristics

Variables	HIV + N=96 n (%)	HIV – N=40 n (%)	P. value
Age			
Mean±SD	39.08±10.22	33.28±8.68	0.002
Range			
[18-28]	14 (14.6)	11 (27.5)	0.006
[28-38]	24 (25.0)	18 (45.0)	
[38-48]	40 (41.7)	8 (20.0)	
≥48	18 (18.8)	3 (7.5)	
Educational level			
None	3 (3.1)	0 (0.0)	0
Primary	42 (43.8)	10 (25.0)	
Secondary	40 (41.7)	21 (52.5)	
High level	11 (11.5)	9 (22.5)	
Marital status			
Married	30 (31.2)	11 (27.5)	0.251
Single	48 (50.0)	26 (65.0)	
Divorced	4 (4.0)	0 (0)	
Widow	14 (14.6)	3 (7.5)	
Occupation			
Housewife	44 (45.8)	19 (47.5)	0.264
Teacher	8 (8.3)	2 (5.0)	
Student	11 (11.5)	11 (27.5)	
Trader	12 (12.5)	6 (15.0)	
Nurse	2 (2.1)	2 (5.0)	
Hairdresser	14 (14.6)	0 (0)	
Restaurateur	5 (5.2)	0 (0)	

Table 2 Distribution of HIV positive women with respect to type of HIV

Type of HIV	Frequency	Parentage (%)
HIV 1	92	95.8
HIV 2	4	4.2
Total	96	100

Regarding the use of condoms, 56.2% of HIV-positive women did not systematically use condoms compared to 37.5% among controls. In contrast, the group infected with HIV had less history of STIs (39.6%) than the control group (67.5%) (Table 3).

Table 3 Distribution of population with respect to behavioural risks and history of STIs

Variables	HIV +	HIV -	P. value
	N = 96 n(%)	N = 40 n(%)	
Number of partners			
None	26 (27.1)	14 (35.0)	
One	58 (60.4)	26 (65.0)	
Two	2 (2.1)	0 (0.0)	
Many	10 (10.4)	0 (0.0)	0
Use of condom			
Yes	32 (33.3)	18 (45.0)	
No	54 (56.2)	15 (37.5)	
Sometimes	10 (10.4)	7 (17.5)	0.126

Variables	HIV +	HIV -	P. value
	N = 96 n(%)	N = 40 n(%)	
History of STIs			
Chlamydia	14 (14.6)	7 (17.5)	0.668
Syphilis	16 (16.7)	7 (17.5)	0.906
Others*	8 (8.3)	13 (32.5)	0

*: infections with trichomonas, gardnerella vaginalis, and candida vaginalis

Biological data

The prevalence of mycoplasma carriage was higher in the controls (62.5%) than in the seropositive (58.3%) with, however, a non-significant statistical difference (P=0, 652) (Table 4).

However, peak frequency of carriage and infection were observed in the age group 18-28 in both groups and HIV+women in this age group were all infected with a mycoplasma. The co-infection rate was 32.3% in cases (HIV+women) and 40% in controls (Table 5).

Table 4 Prevalence of mycoplasma in the study population

	HIV +	HIV -	OR (CI : 95%)	P. value
	N=96 n (%)	N=40 n (%)		
Absence	40 (41.7)	15 (37.5)	1.19 (0.56-2.54)	0.652
Total carriage	56 (58.3)	25 (62.5)	0.84 (0.39-1.79)	0.652
Colonisation	4 (4.2)	2 (5.0)	0.83 (0.15-4.70)	1
Infection with M. hominis	2 (2.1)	0 (0.0)	-	-
Infection with U urealyticum	19 (19.8)	7 (17.5)	1.16 (0.45-3.03)	0.757
Co-infection	31 (32.3)	16 (40.0)	0.72 (0.33-1.54)	0.389

Table 5 Distribution of prevalence rate of mycoplasma in the population according to age

	[18-28]	[28-38]	[38-48]	≥48	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
HIV+	N=14	N=24	N=40	N=18	N=96
Absence	0(0.0)	12(50.0)	20(50.0)	8(44.4)	40(41.7)
Colonisation	0 (0.0)	0 (0.0)	4 (10.0)	0 (0.0)	4 (4.2)
Infection with M. hominis	0 (0,0)	0 (0,0)	2 (5,0)	0 (0,0)	2 (2,1)
Infection with U. urealyticum	4 (28.6)	6 (25.0)	3 (7.5)	6 (33.3)	19 (19.8)
Co-infection	10 (71.4)	6 (25.0)	11 (27.5)	4 (22.2)	31 (32.3)
Sub-total carriage	14 (100.0)	12 (50.0)	20 (50.0)	10 (55.5)	56 (58.3)
Sub-total infected women	14 (100.0)	12 (50.0)	16 (40.0)	10 (55.5)	52 (54.1)
HIV-	N=11	N=18	N=8	N=3	N=40
Absence	1 (9.1)	8 (44.4)	5 (62.5)	1 (33.3)	15 (37.5)
Colonisation	1 (9.1)	1 (5.6)	0 (0.0)	0 (0.0)	2 (5.0)
Infection with M. hominis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Infection with U. urealyticum	3 (27.3)	3 (16.7)	1 (12.5)	0 (0.0)	7 (17.5)
Co-infection	6 (54.5)	6 (33.3)	2 (25.0)	2 (66.7)	16 (40.0)
Sub-total carriage	10 (91.0)	10 (55.5)	3 (37.5)	2 (66.7)	25 (62.5)
Sub-total infected women	9 (81.8)	9 (50.0)	3 (37.5)	2 (66.7)	23 (57.5)

In the search for a possible correlation between the carriage of mycoplasmas and certain socio-demographic parameters in the two groups, it appeared that the prevalence of carriage of mycoplasmas was higher among housewives and single people of the two groups and women of primary educational level were more infected in HIV positive patients: 44.6% (versus 20% in controls) (Table 6).

Table 6 Distribution of prevalence rate of mycoplasma carriers with respect to marital status, type of occupation and level of education

Status	HIV+		HIV-	
	Presence N=56 n(%)	Absence N=40 n(%)	Presence N=25 n(%)	Absence N=15 n(%)
Type of occupation				
Housewife	28 (50.0)	16 (40.4)	11 (44.0)	8 (53.3)
Teacher	2 (3.6)	6 (15.0)	2 (8.0)	0 (0)
Student	11 (19.6)	0 (0.0)	9 (36.0)	3 (20.0)
Trader	4 (7.1)	8 (20.0)	2 (8.0)	3 (20.0)
Nurse	2 (3.6)	0 (0)	1 (4.0)	1 (6.7)
Hairdresser	4 (7.1)	10 (25.0)	0 (0)	0 (0)
Restaurateur	5 (8.9)	0 (0.0)	0 (0)	0 (0)
Marital status				
Married	14 (25.0)	16 (40.0)	4 (16.0)	7 (46.7)
Single	32 (57.1)	16 (40.0)	20 (80.0)	6 (40.0)
Divorced	2 (3.6)	2 (5.0)	1 (4.0)	2 (13.3)
Widow	8 (14.3)	6 (15.0)	0 (0)	0 (0)
Level of education				
None	1 (1.8)	2 (5.0)	0 (0)	0 (0)
Primary	25 (44.6)	18 (45.0)	5 (20.0)	5 (33.3)
Secondary	22 (39.3)	17 (42.5)	14 (56.0)	7 (46.7)
High level	8 (14.3)	3 (7.5)	6 (24.0)	3 (20.0)

Ureaplasma urealyticum had a high sensitivity to macrolides (Figure 3) and josamycin presented a better sensitivity in co-infection situation (Figure 4). All the HIV+ subjects in our study with less than 200 CD4 were carriers of *Ureaplasma urealyticum* (i.e. 100%).

In subjects with a CD4 count >200, 55.4% had a mycoplasma. However, the chi-square correlation test (P=0.094) was not significant. So, the carriage of mycoplasmas was not influenced by the immune state (Table 7).

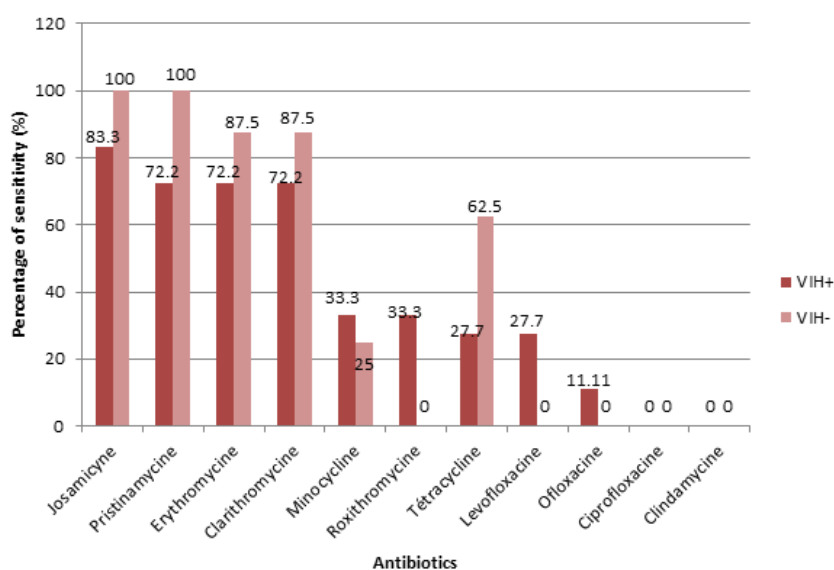


Figure 3 Sensitivity profile of *Ureaplasma urealyticum*.

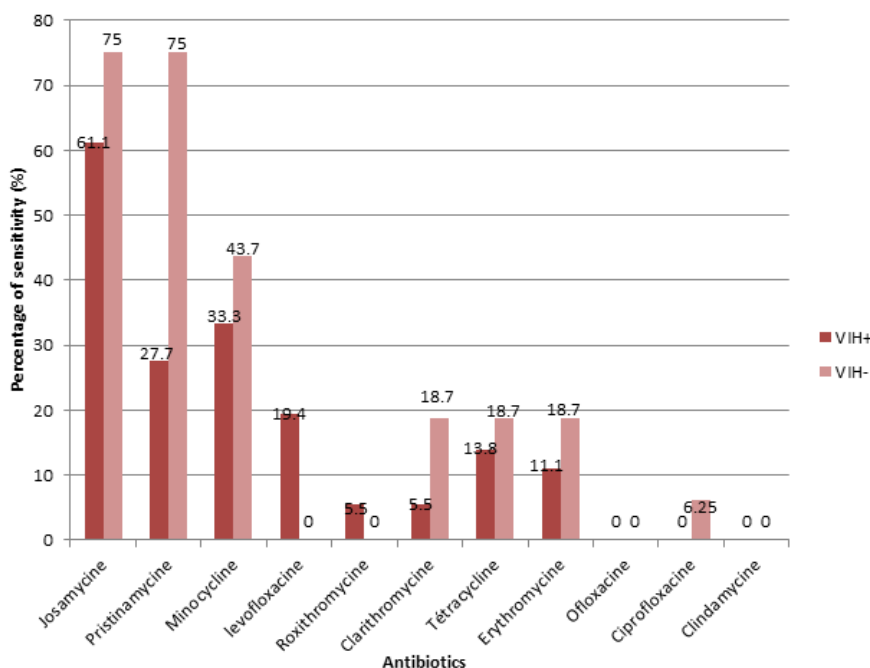


Figure 4 Sensitivity profile of Mycoplasma hominis associated with Ureaplasma urealyticum.

Table 7 Comparison of CD4 count with germs detected

CD4 count	N=96	Mycoplasma hominis n (%)	Ureaplasma urealyticum n (%)	M. hominis et U. urealyticum n (%)	P.Value
≤ 200	6	0 (0.00)	1 (16.7)	5 (83.3)	0.094
200 and above	90	2 (2.2)	17 (18.8)	31 (34.4)	

Discussion

We discussed our results with those of works from the same geographical area and elsewhere

Limits: The declarative nature of the socio-demographic, clinical data and the size of our sample constituted its limits.

Sociodemographic characteristics

The age: The age extremes of our total sample were 18 and 65 with averages of 39.08±10.22 years in HIV positive women and 33.28±8.68 years in HIV negative women. So, we had a sample of women, mostly of reproductive age. Our results approximate the 37 years of Mbamba,⁶ and the 34.02±6.51 years of Djigma⁴ whose extremes were from 20 to 53 years.

Type of occupation, marital status and level of study

Housewives and singles were in the majority in our two matched groups. They had primary education level (41.7%) for HIV positive and secondary education (52.5%) for controls. Our results confirm those of the work of Djigma⁴ and Mbamba.⁶

Prevalence of mycoplasmas

The rate of mycoplasma carriage was 58.3% in HIV-positive women and 62.5% in HIV-negative women. The data in the literature vary widely depending on the place of study, with prevalence varying from 20 to 92%. Rapelanoro et al.⁵ report 92% of mycoplasma

carriage rates in HIV-positive women of reproductive age in Bangui; Faye-kette et al.⁷ in Ivory Coast report carryings of 22% for *U. urealyticum* and 20% for *M. hominis*; 30.9% carriage of *M. hominis* found by Mamadou et al.⁸ in a group of sex workers in Niamey and 71.4% carriage of mycoplasma in Mbamba et al.⁶ in Cameroon in a study carried out on 84 HIV positive women. In our study, we did not observe any statistical difference with respect to HIV status.

The group of 18-28 years old is recognized sexually very active and carefree about STI prevention concerns; this is demonstrated by the high prevalence of mycoplasma infections found in this group in the two groups in our sample, as well as the high rate of risky sexual behavior exhibited by most of these women. All HIV positive women in this age group were infected with *U. urealyticum*.

Sensitivity profile

Macrolides are usually very active on mycoplasmas; evidenced by the high sensitivity of *Ureaplasma urealyticum* and *Mycoplasma hominis* to josamycin and pristinamycin found in our study. Our findings agree with data from the literature and the work of Mbamba⁶ whose strains of mycoplasmas had a sensitivity of 100% to josamycin and pristinamycin.

Correlation of mycoplasmas with the level of immunity

In our study, there was no causal link between CD4 count and mycoplasma infection. The data in the literature are mixed on this subject.

As much as our findings confirm those of Lanzafame³ and Djigma,⁴ they contradict those of Levine⁹ whose work reports a strong association between CD4 count and mycoplasma infection. But this discrepancy in the data is to be credited to the small size of our sample, opposed to its own, but also to its mode of recruitment, which specifically included cases of vaginosis or risky sexual contact with an infected partner. The follow-up and the quality of improved care for HIV-positive women also seem to us to be an argument in favor of this lack of link between CD4 count and mycoplasma infection.¹⁰⁻²⁰

Conclusion

Most HIV-positive women in our sample were infected with mycoplasmas, but without statistical significance between the two groups regarding HIV status. Most HIV positive women had a CD4 count greater than 200. Furthermore, there was no correlation between mycoplasma infection and CD4 count.

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None.

Author contribution

Essome designed the study, collected the data and wrote the manuscript; Nida, Mve, Ekono, Nana, Boten, Tocki have read and corrected the manuscript; Halle and Adiogo supervised the study, corrected and validated the final version of the manuscript.

Conflicts of interest

The authors declare on their honor that they have no conflict of interest.

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