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Epidemiology, Prevalence and Antimicrobial Susceptibility of Sexually Transmitted *Mycoplasma hominis* and *Ureaplasma urealyticum* Infections in Dschang, West Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TDT and ILSN conceived and design the study. Authors ILSN, RHT, CTT, DNS, ACNS and TDT were responsible for data collection and statistical analyzes. Authors TDT, FRNK, HDM, ACNS and ILSN wrote the first draft. All the authors were involved in the critical review of the first draft. All authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

Background: Genital Mycoplasmas play a key etiological role in several urogenital diseases among both Men and Women. Just to mention the few, they are often responsible of non-gonococcal urethritis, spontaneous abortion, preterm birth, low birth weight, infertility and perinatal mortality. **Aim:** The study aimed to assess the prevalence of genital infections with *Mycoplasma hominis, Ureaplasma urealyticum* and their co-infection, as well as the susceptibility profiles to antibiotics commonly prescribed in Dschang, Cameroon.

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Study Design: This was a 5-years retrospective study (including data from January 1, 2015 to December 31, 2019) conducted at Saint Vincent de Paul Hospital in Dschang, Cameroon.

Methods: Data of 338 participants received at the Gyneco-obstetric and Internal Medicine units, properly recorded, were collected from the Hospital Microbiology Laboratory records.

Results: The overall prevalence of genital Mycoplasmas was 57.4%, with 44.4% attributed to *Ureaplasma urealyticum*, 5.9% to *Mycoplasma hominis* and 7.1% to the Co-infection with the two bacteria isolates. Josamycin was the most sensitive antibiotic against *Ureaplasma urealyticum* (85.3%) and the co-infection (91.7%). *Mycoplasma hominis* isolates were more susceptible to three fluoroquinolones and tetracyclines with an equal sensitivity rate of 80.0%. Emphasis is on the increasing resistance of co-infection towards Macrolides (83.3%) in this locality. Summarily, Josamycin was the antibiotic to which genital Mycoplasmas showed the lowest resistance rate (6.2%) while the highest (62.9%) was attributed to Acetylspyramycin.

Conclusion: The implementation of health policies in Cameroon should optimize on mechanisms of diagnosis, proper treatment and monitoring the antibiotic resistance of commonly isolated genital Mycoplasmas, regarding their impact on reproductive health.

Keywords: Mycoplasma hominis; Ureaplasma urealyticum; genital infection; antimicrobial susceptibility; epidemiology.

1. INTRODUCTION

Mvcoplasma hominis and Ureaplasma urealyticum. commonly known as genital Mycoplasmas are small ubiquitous bacteria. devoid of cell-wall and capable of a free life [1]. Their detection in routine practice is done by culture and biochemical characterization in liquid media [2,3] and by the polymerase chain reaction [4]. These organisms play an etiological role in several urogenital diseases among both Men and Women [5]. These include infections, such as pelvic inflammatory diseases, pyelonephritis, endometritis, chorioamnionitis and postpartum fevers. These conditions lead to significant complications such as spontaneous abortion, preterm birth, low birth weight, infertility, respiratory infections of the newborn and perinatal mortality [6-9]. U. urealyticum and M. hominis are one of the causes of non-gonococcal urethritis (NGU) in humans [10,11].

Despite the fact that Mycoplasmas are found in the genital tract of 5-20% of sexually active Men and 40-80% of sexually active women, the genital carriage of these microorganisms is directly related to the number of sexual partners in adults [1]. The epidemiology of Mycoplasma infections remains a subject of interest in infectious disease worldwide. Hence, research outcomes are quite considerable depending on the socio-economic context and the population concerned [12]. To mention a few, a prevalence of 31.2% for U. urealyticum and 0.7% for M. hominis were reported in a cohort of women in China [13], 9.0% and 8.6% for U. urealyticum and *M. hominis* respectively among child-bearing age women in Italy [14]. An overall prevalence of

39.7% was obtained among pregnant women in South Africa [15], and 45.4% among women of childbearing age in Ndjamena in Thad [16]. Fouzia et al in Morocco reported prevalences of 36% and 21% respectively among Men and Women with controlled sexual behaviors, compared to 41% and 59% in the same order among Men and Women with uncontrolled sexual behaviors [17].

Although early detection of genital Mycoplasma and other sexually transmitted infections (STIs) is the best option for preventing infertility [18], the state of act of these infections in Cameroon remains very sporadic and restricted to two major Cities. Prevalences of genital Mycoplasmas of 65.0% [19] and 38.0% [20] were respectively reported among pregnant women in Yaoundé and Douala. Gonsu also obtained a prevalence of 71.4% among HIV/AIDS-positive pregnant women in Yaoundé [21]. The prevalences of 10.8% and 89.2% recorded respectively among Male and Female genders in Yaoundé, are the only results generated from the general population in Cameroon [22]. No prevalence has yet been mentioned in the West region of Cameroon and more specifically in the community of Dschang.

The susceptibility profile of genital mycoplasma common antimicrobials has been to described from several angles by various Waites and Talkington authors. present fluoroquinolones tetracyclines, and MLSK (Macrolides, Lincosamides, Streptogramins and Keltolides) group of antibiotics as drugs with better activity against genital Mycoplasmas [11]. Ciprofloxacin and Ofloxacin were listed among the six recent fluoroquinolones with good susceptibility against these organisms, while Norfloxacin and cinoxacin were among the inactive [23]. According to the World Health Organization, antimicrobial resistance is a growing phenomenon worldwide with everincreasing prevalences threatening human health [24]. In Poland, Ureaplasma demonstrated increasing resistance to Clarithromycin (from 0 to 20% between 2003 and 2015) while M. hominis exhibited intrinsic resistance to macrolides such as: Erythromycin, Clarithromycin and Azithromycin [25].

The National Centre for Sexually Transmitted Bacterial Infections has reported a resistance rate of 5.0% to tetracycline by M. hominis and 5.4% to levofloxanine by U. uréalvticum in 2018 in France [26]. In a population of pregnant women in Douala, U. urealyticum was resistant to Clindamycin and *M. hominis* to Erythromycin, Tetracycline and Macrolides [20]. Njunda et al. also reported a strong resistance to Lincomycin and Ciprofloxacin during co-infections [19]. One of the most significant resistance (92.0%) of genital Mycoplasmas was observed with Cotrimoxazole. The respective resistance rates of 85.7% and 92.9% of U. urealyticum and coinfection to Ciprofloxacin, 100% of M. hominis to Roxythromycin were reported within the general population in Yaoundé [22]. Apart from the statistics of the cities of Douala and Yaoundé above mentioned, data on antibiotic resistance of urogenital Mycoplasmas from the rest of the Cameroon territory were not found in any literature consulted.

Given the established risks and complications of aenital Mycoplasmas in Obstetrics and Gynecology, and the increasingly reported antibiotic resistance underlying, we find it relevant to establish the state of act of this infection in the Dschang community in order to provide additional data, crucial for proper assesment at the national level and beyond. The objective of this study was to determine the prevalence of genital infections with M. hominis, U. urealyticum and co-infection to these two germs in Dschang, while emphasizing on their susceptibility profiles to commonly prescribed antibiotics.

2. MATERIALS AND METHODS

2.1 Participants

The present study included a total of 338 participants of both gender received in the

Gyneco-obstetric and Internal Medicine units of the Saint Vincent de Paul Hospital (SVPH) in Dschang, suspected by a clinician of symptomatic carriage of genital Mycoplasmas infection.

2.2 Study Design and Duration

A retrospective study was conducted between July and September 2020 including data obtained over a period of 5 years, ranging from January 1, 2015 to December 31, 2019. Data from the 338 properly registered participants were collected (Age, gender, Mycoplasmas culture result and susceptibility profile) from the SVPH Microbiology Laboratory Records.

The Mycoplasma IES test kit (Zhuhai Encode Medical Engineering, Ltd.) was the one used in this Laboratory for the cultivation, identification, quantification and susceptibility of M. hominis and U. urealyticum in the genital samples of participants according to the standard operating procedure of the analysis. Thus the isolated bacteria and their respective susceptibility to the following 12 antibiotics: Doxycicline (DOX). Minocycline (MIN), Ciprofloxacin (CIP), Ofloxacin Sparfloxacin (SPA), Roxithromycin (OFL), (ROX), Azithromycin (AZI), Clarithromycin (CLA), Josamycin (JOS), Spectinomycin (SPE), Acetylspyramycin (ASP) and Levofloxacin (LEV) were each time documented.

2.3 Study Area

The SVPH is a reference Hospital in the West Region of Cameroon. It is located in the Dschang Health District which is the largest Health District of the Region, covering an average population of 232,326. The city of Dschang, capital of the Menoua Division is located in the south-west of Mount Bamboutos (Fig. 1) between 5-10 and 5-38' northen latitudes and 9-50 and 10-20 degrees east longitude, at an altitude of 1400m above the sea level. With a capacity of 97 beds, the SVPH is made up of specialized units such Surgery, Ophthalmology. Oto-rhinoas: laryngology, Gyneco-Obstetrics, Paediatrics, Dental Care, Medical Imaging, Mental Health, and Medical Laboratory units. The Hospital also work in care and treatment of malaria, tuberculosis and HIV/AIDS through the Drug Research Enhancement Against AIDS and Malnutrition (DREAM) convention signed with the Saint Egidio Community, and recently in the molecular diagnosis and management of the new COVID-19 cases.

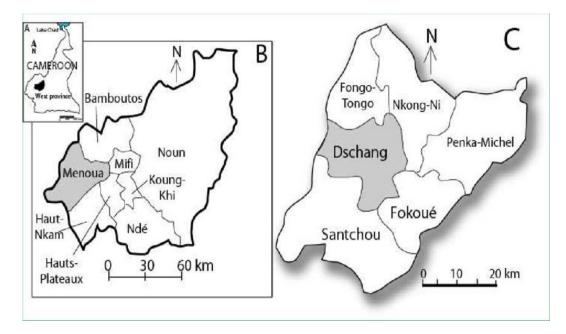


Fig. 1. Geographical location of the city of Dschang: In Cameroon (A), in the west region (B) and in the Menoua Division (C) [27]

2.4 Statistical Analysis

Data were registered using the Microsoft Excel 2012 spreadsheets and transferred to IBM SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA) for analysis. Results expressed in terms of proportions and frequencies are presented in tables using Microsoft Word 2012 software; and figures using Microsoft Excel 2012 software. Prevalence specific to each characteristic and medians with their percentiles (25 - 75) were calculated and presented in tables. Chisquare (χ^2) or Fisher's exact tests were used to compare and evaluate the association between age, gender and occurrence of genital Mycoplasmas infection for a 5% significance level (p<0.05).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Socio-demographic characteristics

Out of the 338 participants, female represented of 72.8% (250/338) against 27.2% (88/338) of male gender giving a sex ratio of 2.68 in favor of the Female. Participant's age ranged from 18 to 70 years with a median age of 28 years (interquartile: 25 - 33). The most represented age group was 18-29 years old with a frequency of 55.1% for a total of 75 participants, while those aged 49 years and above where the least represented with 3.6% (Table 1).

3.1.2 Prevalence of genital mycoplasmas

Results showed an overall positivity to genital Mycoplasmas of 57.4% (194/338), with 44.4% (150/338) attributed to U. urealyticum, 5.9% (20/338) to M. hominis and 7.1% (24/339) to the co-infection (U. uréalyticum + M. hominis). Although age was not associated with the occurrence of the disease (P = .29), the most affected age group was 29-39 years with a 66.1% prevalence (82/124), and that of those aged 49 and above the less affected with 16.7% (2/12). The female gender was more infected with 64.0% of cases (160/250) as compared to the 38.6% (34/88) obtained from the Male The difference was statistically gender. significant (P = .03) (Table 2). There was a significant oscillation in the prevalence of these pathogens throughout the 5 years, with the highest prevalence of 80.0% observed in 2017 (Fig. 2).

3.1.3 Antimicrobial susceptibility pattern

The susceptibility of *M. hominis, U. urealyticum* and co-infection to the twelve antimicrobials mentioned above was equally reported using the Mycoplasma IES test Kit. *U. urealyticum* showed a greater sensitivity to Josamycin (85.3%), Sparfloxacin (85.3%) and Clarithromycin (80.0%) was observed while the lowest with Acetylspyramycin (12.0%). M. hominis was more sensitive to the three Fluoroquinolones (i.e. Levofloxacin, Ofloxacin and Sparfloxacin) and Tetracyclines (i.e. Doxycicline and Minocycline) with an equal sensitivity rate of 80.0% for these antibiotics. The same bacterium demonstrated an increased resistance to Roxithromvcin (70.0%). While co-infection with both Mycoplasmas was better treated with Josamycin (91.7%), a greater resistance of these two germs (83.3%) was observed towards three Macrolides: Acetylspyramycin, Azithromycin, Roxithromycin (Table 3).

The assessment of the overall susceptibility of genital Mycoplasmas in the 5 recent years shows that Josamycin has been the antibiotic to which genital Mycoplasmas had the lowest resistance (6.2%) while the highest (62.9%) was attributed to Acetylspyramycin (Table 4).

The sensitivity of genital Mycoplasmas to the various antibiotics tested over time shows that Sparfloxacin was the most sensitive drug in 2015 with a sensitivity of 100% while Levofloxacin was the most sensitive in 2016 with a sensitivity rate of 86.4% (with 38 sensitive pathogens out of a total of 44 isolated that year). Josamycin was the molecule with the highest sensitivity in 2017 and 2018 with frequencies of 95.0% and 100% respectively. The results also show a significant decrease in the sensitivity of almost all antibiotics in the years 2018 and 2019 from 23.7% in 2017 to 21.1% and 21.6% in 2018 and 2019 respectively (Fig. 3).

3.2 Discussion

Genital Mycoplasma infections are among the STIs frequently incriminated in human infertility

[18]. They remain a real health threatening issue even in communities of average standard of living in Cameroon [19]. The present study conducted in Dschang reported a prevalence of genital Mycoplasmas of 57.4% (194/338), with 44.4% (150/338) attributed to U. urealyticum, 5.9% (20/338) to M. hominis and 7.1% (24/339) to their co-infection. Ahouga et al. achieved a similar overall prevalence (58.73%) in the general population in Yaoundé [22]. These rates are less than 65% presented among pregnant women in the same city [19]. This disparity be justified can by the difference between the populations studied. While their study focused on a population of pregnant women who are naturally immunosuppressed by their physiological condition, ours focused on a healthy population in the absence of any other disease condition. However, these prevalences in Cameroon are still higher than those reported in other studies in Poland, in the United States and China, which had lower prevalences respectively of 12.9% [25], 21.4% [28] and 33.8% [13]. This disparity resides in the difference of the socio-economic context that prevails at the global level. While the conditions of developed countries promote access to quality health care but also an anticipation of the disease and better care, Africa and Cameroon in particular remain far from that reality.

Despite the availability of susceptible antibiotics to the genital Mycoplasma species isolated throughout the 5 years, there has been a continuous oscillation of high prevalence of these pathogens, with the highest prevalence of 80.0% observed in 2017. This is probably due to sexual practice of unprotected promiscuity. sexual non-treatment of intercourse and sexual partners.

Characteristics	Number of participants n (%)	U. urealyticum n (%)	M. hominis n (%)	Co- infection n (%)	Total positive n (%)	P value
Age (in Years)						
[18-29]	174 (51.5)	64 (36.8)	14 (8.0)	14 (8.0)	92 (52.9)	
[29-39]	124 (36.7)	68 (54.8)	6 (4.8)	8 (6.5)	82 (66.1)	.29
[39-49]	28 (8.3)	16 (57.1)	0 (0.0)	2 (7.1)	18 (64.3)	
[49- +]	12 (3.6)	2 (16.7)	0 (0.0)	0 (0.0)	2 (16.7)	
Gender						
Male	88 (26.0)	28 (31.8)	2 (2.3)	4 (4.5)	34 (38.6)	.03
Female	250(74.0)	122 (48.8)	18 (5.6)	20 (8.0)	160 (64.0)	
Total	338 (100)	150 (44.4)	20 (5.9)	24 (7.1)	194 (57.4)	

n(%) = Frequency (percentage); Co-infection = U. urealyticum + M. hominis

Antibiotics	Ure	eaplasma ureal (N=150)	yticum	N	lycoplasma ho (N=20)	minis		Co-Infection (N=24)	n
	S		R	S		R	S		R
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Aminoside									
Spectinomycin	30(20.0)	72(48.0)	48(32.0)	14(70.0)	2(10.0)	4(20.0)	2(8.3)	8(33.3)	14(58.3)
Fluoroquinolones									
Ciprofloxacin	38(25.3)	92(61.3)	20(13.3)	12(60.0)	4(20.0)	4(20.0)	2(8.3)	12(50.0)	10(41.7)
Levofloxacin	106(70.7)	36(24.0)	8(5.3)	16(80.0)	0(0.0)	4(20.0)	8(33.3)	8(33.3)	4(33.3)
Ofloxacin	49(65.3)	21(28.0)	5(6.7)	8(80.0)	0(0.0)	2(20.0)	3(25.0)	10(41.7)	4(33.3)
Sparfloxacin	128(85.3)	14(9.3)	8(5.3)	16(80.0)	4(10.0)	2(10.0)	12(50.0)	4(33.3)	2(16.7)
Macrolides/Lincosa	mides		• •						
Acetylspyramycin	8(12.0)	42(28.0)	90(60.0)	4(20.0)	4(20.0)	12(60.0)	2(8.3)	2(8.3)	10(83.3)
Azithromycin	64(2.7)	72(48.0)	14(9.3)	4(20.0)	4(20.0)	12(60.0)	0(0.0)	4(16.7)	10(83.3)
Clarithromycin	120(80.0)	14(9.3)	16(10.7)	2(10.0)	6(30.0)	12(60.0)	4(16.7)	10(41.7)	5(41.7)
Josamycin	128(85.3)	14(9.3)	8(5.3)	14(70.0)	2(10.0)	4(20.0)	22(91.7)	2(8.3)	0(0.0)
Roxithromycin	26(17.3)	72(48.0)	52(34.7)	2(10.0)	4(20.0)	14(70.0)	4(16.7)	0(0.0)	10(83.3)
Tetracyclines	• •	· ·	· · ·	· · ·	· ·	• •	· ·	• •	
Doxycycline	114(76.0)	14(9.3)	22(14.7)	16(80.0)	4(20.0)	0(0.0)	18(75.0)	4(16.7)	2(8.3)
Minocycline	106(70.7)	20(13.3)	24(16.0)	16(80.0)	4(20.0)	0(0.0)	10(41.7)	10(41.7)	4(16.7)

Table 2. Susceptibility profile of genital mycoplasmas to antibiotics

S= Sensitive; I= Intermediate; R= Resistant; n= Number; Co-infection = M. hominis + U. urealyticum

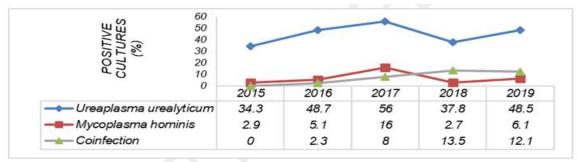


Fig. 2. Trend of prevalence of genital mycoplasmas between 2015 and 2019

Antibiotics	Sensitive	Intermediate	Resistant n (%)	
	n (%)	n (%)		
Aminoside				
Spectinomycin	46 (23.7)	82 (42.3)	66 (34.0)	
Fluoroquinolones	· · · · ·	· · ·		
Ciprofloxacin	52 (26.8)	108 (55.7)	34 (17.5)	
Levofloxacin	130 (67.0)	44 (22.7)	20 (10.3)	
Ofloxacin	120 (61.9)	52 (26.8)	22 (11.3)	
Sparfloxacin	156 (80.4)	24 (12.4)	14 (7.2)	
Macrolides/Lincosamides	;			
Acetylspyramycin	24 (12.4)	48 (24.7)	122 (62.9)	
Azithromycin	68 (35.1)	80 (41.2)	46 (23.7)	
Clarithromycin	126 (64.9)	30 (15.5)	38 (19.6)	
Josamycin	164 (84.5)	18 (9.3)	12 (6.2)	
Roxithromycin	32 (16.5)	76 (39.2)	86 (44.3)	
Tetracyclines				
Doxycycline	148 (76.3)	22 (11.3)	24 (12.4)	
Minocycline	132 (68.0)	34 (17.5)	28 (14.4)	
Decreasing order of S	Sensitivity : JOS, SPA, DOX, MI	N, LEV, CLA, OFL, AZI, CIP, S	SPE, ROX, ASP	

Table 3. Global susceptibility of genital mycoplasmas to antibiotics

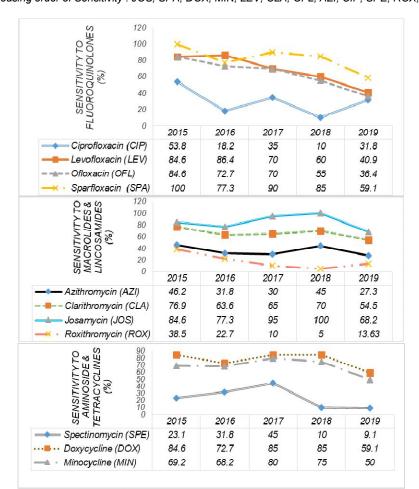


Fig. 3. Trend of sensitivity of *M. hominis* and *U. urealyticum* isolates to fluoroquinolones, macrolides/lincosamides and aminoside/tetracyclines between 2015 and 2019

Though age was not associated with the occurrence of the disease, the age group most affected was 29-39 years with a prevalence of 66.1% (82/124) and that of those aged 49 and above less affected with 16.7% (2/12). While Wang et al. also found the highest prevalence (49.7%) in the 30-39 age group [13], Imudia et al. demonstrated that nearly two-thirds of M. hominis and U. urealyticum infections were found in the same age group (30-39 years old) [28]. On the other hand, other authors revealed in the results of their studies a predominance of these pathogens rather in the lower age group (25-29 years) [2,19]. However, the point of convergence of all these studies remains that genital colonization by Mycoplasmas is much more recurrent in people of childbearing age.

The female gender was more infected with 64.0% of cases (160/250) compared to 38.6% (34/88) attributed to the male gender with a statistically significant difference between these two groups (P = .03). This is probably because of the Female anatomy which predisposes her to a variety of gynecological infections compared to Male. Similarly, a study conducted in Morocco revealed respective prevalencies of 59% and 41% respectively among Female and Male genders [17]. The capability of these pathogens to reside as commensals increases the chances of developing genito-urinary tract infections, especially in individuals with multiple sexual partners. This therefore calls for better follow-up of women in order to reduce the adverse fetal outcome and the continuous increasing infertility rate in couples.

In the present study, Genital Mycoplasmas showed great variability in the susceptibility profile to the antibiotics tested. The greatest sensitivities of U. urealyticum were observed with Josamycin (85.3%), Sparfloxacin (85.3%) and Clarithromycin (80.0%) Respectively. Bayraktar et al. also had satisfactory results for Josamycin (92.6%) and Clarithromycin (88.9%) which were among the most active antimicrobials against the bacterium [2]. M. hominis was more sensitive to the three fluoroquinolones (i.e. Levofloxacin, Ofloxacin and Sparfloxacin) and to Tetracyclines (i.e. Doxycicline and Minocycline) with an equal sensitivity rate of 80.0% for these antibiotics. In contrast to the high sensitivity of fluoroquinolones observed in our study, Wang et al. rather obtained an opposite effect where this class of antibiotic was among the less active in the treatment of infections with *M. hominis* [13]. The agreement with their study lies in the sensitivity to Tetracyclines where they even obtained more convincing results (up to a 100%) in this class of antibacterials. Increased resistance of M. hominis to Roxithromycin (70.0%) has also been reported by several other authors [19,29]. The co-infection with both Mycoplasmas was best treated with Josamycin (91.7%). This result is also similar to that of Wang et al. [13] who presented satisfactory performances of this antibiotic (73.0%) during co-infections. Increased resistance (83.3%) of co-infection by these two germs vis-à-vis macrolides (Acetylspyramycin, Azithromycin and Roxithromycin) was also observed (up to 90.0%) by Nuradilova et al. in 2019 [30]. The comparison of the results of this study with current global knowledge on the topic denotes similarities and contrasts in the susceptibility profile of urogenital Mycoplasmas to the various antibiotics tested. The highlight remains an urgent need to have, as much therapeutic options as possible in order to hope to overcome the multiple antimicrobial resistance that is fast coming in the years ahead [24]. The order of susceptibility of the 12 antibiotics tested in this study places Josamycin at the forefront of sensitivity. This result is similar to those of other authors [2,13,19] where it was also ranked among the most actives regading its antibiotic activity towards Mycoplasmas. These results demonstrate with reserve that Josamvcin remains an antibiotic of great interest in **Mvcoplasmas** infections. The significant decrease in the sensitivity of almost all antibiotics in the years 2018 and 2019 (from 23.7% in 2017 to 21.1% and 21.6% in 2018 and 2019 respectively) reiterates the urgent need to develop and implement the national action plan to counteract antimicrobial resistance as prescribed by WHO in the Global Action Plan on Antimicrobial Resistance [24].

4. CONCLUSION

Results the study showed an overall prevalence of genital Mycoplasmas of 57.4%, with 44.4% attributed to U. urealyticum, 5.9% to M. hominis and 7.1% to the co-infection. Based on recent studies results, these prevalence are among the highest in Cameroon. There is a great variability in susceptibility profile of the 12 antimicrobials tested. In the last 5 years, Josamycin has been the antibiotic to which genital Mycoplasmas showed the lowest resistance (6.2%) while the highest (62.9%) is attributed to Acetylspyramycin. Emphasis is raised on increasing resistance of co-infection towards Macrolides (83.3%) in this locality. Given the impact of these microorganisms on reproductive health, it is important to pay special attention by maximizing the diagnosis in symptomatic patients. Also, the implementation of health policies in Cameroon should optimize on the monitoring mechanisms for antibiotic resistance of commonly isolated genital mycoplasmas.

ETHICAL APPROVAL

The study was examined and approved by the administrative authorities of the SVPH dschang.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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