Genital mycoplasmas in women attending the Yaoundé University Teaching Hospital in Cameroon

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Abstract

Genital mycoplasmas are implicated in pelvic inflammatory diseases, puerperal infection, septic abortions, low birth weight, nongonococcal urethritis and prostatitis as well as spontaneous abortion and infertility in women. There is paucity of data on colonisation of genital mycoplasma in women and their drug sensitivity patterns. The aim of our study was to determine the prevalence of genital mycoplasmas (*Ureaplasma urealiticum* and *Mycoplasma hominis*) infection and their drug sensitivity patterns in women. A mycofast kit was used for biochemical determination of mycoplasma infection in 100 randomly selected female patients aged 19-57 years, attending the University of Yaoundé Teaching Hospital (UYTH) from March to June 2010. Informed consent was sought and gained before samples were collected. Genital mycoplasmas were found in 65 patients (65%) [95% CI=55.7-74.3%] and distributed as 41 (41%) [95% CI=31.4-50.6%] for *U. urealiticum* and 4 (4%) [95% CI=0.20-7.8%] for *M. hominis* while there was co-infection in 20 women (20%) [95% CI=12.16-27.84%]. In our study, 57 (57%) [95% CI=47.3-67%] had other organisms, which included *C. albicans* (19 [19%]), *G. vaginalis* (35 [35%]) and *T. vaginalis* (3 [3%]). Among the 65 women with genital mycoplasma, the highest co-infection was with *G. vaginalis* (33.8%). Pristinamycine was the most effective antibiotic (92%) and sulfamethoxazole the most resistant (8%) antibiotic to genital mycoplasmas. We conclude that genital mycoplasma is a problem in Cameroon and infected women should be treated together with their partners.

Introduction

Mycoplasmas are the smallest known free-living organisms with a size between 150 and 250 nm. They are fungi-like in nature, hence the prefix myco-, and lack a cell wall, hence the suffix -plasma. Mycoplasma refers to the plasticity of bacterial forms resembling fungal elements. The absence of a cell wall in mycoplasmas is responsible for the lack of a Gram stain reaction and non-susceptibility to most antimicrobials including beta-lactams, which act on cell walls. They inhabit mucous membranes as in the genital and respiratory tracts.[1] Mycoplasmas were first thought to be viruses because they passed through filters that retain bacteria. It was discovered later that they had bacterial properties (both DNA and RNA and they could grow in cell-free media). They differ from L-form bacteria in that they have sterols in their cell membranes.[2] Genital mycoplasmas have been implicated in pelvic inflammatory diseases, puerperal infection, septic abortions, low birth weight, nongonococcal urethritis and prostatitis as well as spontaneous abortion and infertility.[3] Mycoplasmas are also known to be part of the commensal flora of the genitourinary tract mucosa and are found in the majority of sexually active humans.[4,5] *Mycoplasma genitalium* has become the third most frequent pathogen causing nongonococcal urethritis.[6] It has also been reported that *Mycoplasma hominis* is a cofactor for bacterial vaginosis and pelvic inflammatory disease.[6] Gdoura *et al.*,[7] in a study conducted in Tunisia, have shown a high prevalence of *M. hominis*.

Our study investigated the genital mycoplasma species *M. homonis* and *Ureaplasma urealiticum* and their drug sensitivity patterns. These microorganisms are cultured as compared to other mycolasmas, which are fastidious and cannot be cultured but can only be studied using the PCR method.[8] Our study also investigated the relationship between genital mycoplasma infection and *Candida albicans*, *Gadnerella vaginalis* and *Trichomonas vaginalis*. We report here the prevalence of genital mycoplasmas in women attending the University of Yaoundé Teaching Hospital, their drug sensitivity pattern and the relationship between genital mycoplasmas and *C. albicans*, *G. vaginalis* and *T. vaginalis*.

Materials and Methods

Study site

Our study was carried out in the bacteriology laboratory of the University of Yaoundé Teaching Hospital. The study participants were inhabitants of Yaoundé and its environs. Yaoundé is the capital of Cameroon and is made up of inhabitants from all parts of the country as it is a metropolitan city.

Design and sampling procedures

The design was a cross-sectional one. Sexually active women, both pregnant and non-pregnant, were randomly selected for the study. This study was conducted between March 1 and June 30, 2010. The sample size consisted of patients who attended the University of Yaoundé Teaching Hospital for different obstetric and gynaecological problems. Women who were menstruating, who did douching (vaginal washing) and virgins were all excluded. The patients were educated on the advantages of performing this test and informed consent was sought and gained before samples were collected. If the patient decided to do the test, we proceeded with collection of high vaginal swabs.

Laboratory procedures

Mycofast Evolution 3 Culture Media

Mycofast Evolution 3 Media (BioMerieux) permit the detection, quantification and identification of *M. hominis* and *U. urealiticum* from endocervical, urinary and gastric specimens and in spermatozoa. It also gives the sensitivity of these organisms to different antibiotics. Mycofast Evolution 3 Culture Media is a liquid method based on the ability of *M. hominis* and *U. urealiticum* to metabolise urea and arginine, respectively. The presence of genital mycoplasmas is indicated by a change in colour with the aid of phenol red. This changes the colour of the media from yellow to orange or red owing to the release of ammonia. The reagents used included U.M.M.t: containing transport media for mycoplasma; U.M.M.lyo: with lyophilised

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Key words: genital mycoplasma, women, drug sensitivity pattern, Cameroon.

Conflict of interest: the authors report no conflicts of interest.

Received for publication: 27 October 2010.
Accepted for publication: 20 January 2011.

Journal of Public Health in Africa 2011; 2:e16
doi:10.4081/jphia.2011.e16

Table 1. Prevalence of genital mycoplasmas in the different age groups.

Age range (yr)	Total patients No (%)	*Mycoplasma hominis* (MH) only No (%)	*Ureaplasma urealiticum* (UU) only No (%)	Mixed infection (MH+UU) No (%)	Total No (%)
15-19	2 (2)	0 (0)	1 (1)	1 (1)	2 (2)
20-24	18 (18)	2 (2)	6 (6)	4 (4)	12 (12)
25-29	24 (24)	1 (1)	11 (11)	4 (4)	16 (16)
30-34	18 (18)	1 (1)	5 (5)	4 (4)	10 (10)
35-39	10 (10)	0 (0)	8 (8)	0 (0)	8 (8)
40-44	13 (13)	0 (0)	4 (4)	3 (3)	7 (7)
45-49	10 (10)	0 (0)	3 (3)	3 (3)	6 (6)
50-54	4 (4)	0 (0)	3 (3)	1 (1)	4 (4)
55-59	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Total	100	4 (4)	41 (41)	20 (20)	65 (65)

media for growth; Galerie Mycofast Evolution 3: containing 20 wells; S.M.h: *Mycoplasma hominis* activator (4.5 mL).

Inoculation on media

All reagents were allowed to attain room temperature for 20-30 minutes. The swab was placed in U.M.M.t and mixed. The U.M.M.lyo was regenerated with the entire content of the U.M.M.t medium. Half a millilitre of the latter was transferred into the empty U.M.M.t vial and incubated for 24-48 hours at 37°C. A yellow colouration is negative while a red/orange screen is a positive result. The remaining 2.5 mL of inoculated U.M.M.lyo medium that was stored at 2-8°C was used to continue the procedure for positive results. The diagnosis of the species was confirmed with the Mycofast Evolution Tray 3 and S.M.h. An antibiotic susceptibility test was carried out for each species and read as intermediate, sensitive or resistant.

T. vaginalis was identified using wet preparations. Gram smears of genital specimens were carried out. The specimens were inoculated aseptically on sabouraud supplemented with 5% chloramphenicol agar plates. Plates were incubated at temperatures of 37°C for 24 hours, after which they were examined for colonial characteristics and their identity confirmed and characterised by the following tests: germ tube and biochemical kit analytic profile index (API 20E) for *C. albicans* and *G. vaginalis*.

Data management and analysis

Each patient's demographic profile including laboratory results were entered on structured forms for all clinic days during the study period. The information was verified every week for the use of correct codes and consistency checks. Data were entered into Microsoft Excel sheets and exported to Epi-Info for analysis using descriptive statistics. Statistical significance was set at 95%.

At the initial step of the analyses, frequency distributions of each variable were produced and the information arranged according to age groups and sensitivity patterns. Associations were established between variables of different measures through cross-tabulations. Further analysis included a data summary including proportions, percentages and standard deviations. In these analyses, such methods as the χ^2 test, Fisher exact test for the test of significance of associations between categorical variables, and the student-t test to test statistical significance of hypotheses for continuous variables were used.

Table 2. Reltionship between genital mycoplasmas and *Candida albicans*, *Gadneralla vaginalis* and *Trichomonas vaginalis*.

Microorganisms	Number examined n=100 No (%)	Co-infection with genital mycoplasma n=65 No (%)	Percentage interaction of these pathogens with genital mycoplasmas
Candida albicans	19 (19)	12 (18.5)	63.2
Gadnerella vaginalis	35 (35)	22 (33.8)	62.9
Trichomonas vaginalis	3 (3)	2 (3.1)	66.7
Total	57 (57)	36 (55.4)	63.2

Results

Prevalence of genital mycoplasmas among the different age groups

We studied 100 female patients ranging between the ages of 19 and 57 years. Table 1 shows that genital mycoplasmas were detected in 65 women (65%) [95% CI=55.7-74.3%], 41% [95% CI=31.4-50.6%] had *U. urealiticum*, 4% [95% CI=0.20-7.8%] had *M. hominis* and 20% [95% CI=12.16-27.84%] had mixed infection. Mycoplasma infection was highest in the 25-29 year group (16%) [95% CI=15.7-16.3%].

Interaction between genital mycoplasmas and other pathogens

Out of the 100 female patients who were examined for genital mycoplasmas, 57 (57%) [95% CI=47.3-67%] had other pathogens, which included *C. albicans* (19 [19%]), *G. vaginalis* (35 [35%]) and *T. vaginalis* (3 [3%]). Of the 65 women who had genital mycoplasmas, 36 (55.4%) harboured these other pathogens. These included mainly *G. vaginalis* (22 [33.8%]) followed by *C. albicans* (12 [18.5%]) and *T. vaginalis* (2 [3.1%]). There was a 63.3% interaction of these organisms with genital mycoplasma. There was no significant difference in the rate of interaction with the different pathogens (P>0.5) (Table 2).

Assessment of antibiotics against genital mycolasmas

The mycofast kit for mycoplasma also contained wells for different antibiotics, which included lincomycine, trimethoprim-sulfamethoxazole, erythromycin, doxycycline, pristinamycine, ciprofloxacin, ofloxacine, josamycine, azithromycin and roxithromycin.

Antibiotic susceptibility of *M. hominis*, *U. urealiticum* and co-infection were determined. *U. urealiticum* was very sensitive to erythromycin, pristinamycine and josamycine and least sensitive to lincomycine and ciprofloxacin. *M. hominis* was most sensitive to lincomycine, doxycycline, pristinamycine and josamycine and least sensitive to sulfamethoxazole, erythromycine, roxithromycine and azithromycin (Table 3). The most sensitive antibiotic to geni-

tal mycoplasmas was pristinamycine (92%) while the least sensitive was trimetropine-sulfamethoxazole (8%) (Table 4).

Discussion

Out of the 100 women, 65 were positive for genital mycoplasmas, giving a prevalence of 65% with 4% for *M. hominis*, 41% for *U. urealiticum* and a co-infection of 20%. Mixed infection is owing to the fact that both pathogens survive better in alkaline pH.[9] This is a little higher than results obtained by Elias *et al*.[10] who, in a group of 222 women in a similar age range, found *U. urealiticum* in 31.8% and *M. hominis* in only 3% of the cases. Schlicht *et al*.[11] found a higher prevalence of 54% for *U. urealiticum*. Agbakoba and associates[9] found a prevalence of 36.7% for genital mycoplasmas while working with Nigerian women. Zdrodowska-Stefanow *et al.*,[12] in a similar study in women presenting with urogenital diseases, showed that *U. urealyticum* was detected in 161 (29.8%) and *M. hominis* in 20 (3.7%) women. In this study, the fact that the highest percentage of mycoplasma-positive cultures was found in patients of the STD clinic and in infertile women confirms the fact that mycoplasmas play an important role in the aetiopathogenesis of inflammatory states of the genitourinary organs. It was also observed that genital mycoplasma infection is not specific to any age group but that with the highest prevalence is the 25-30 year group with 16%. It is related to sexual activity, hence any sexually active group is a potential carrier. A similar result was obtained by Zdrodowska-Stefanow *et al.*,[12] with the highest rate in the age range of 26-30 years (29.2% for *U. urealyticum* and 50.0% for *M. hominis*).

Among the women in our study, 57% had other pathogens including *C. albicans* (19%), *G. vaginalis* (35%) and *T. vaginalis* (3%). Thirty-six (55.4%) of these pathogens were co-infected with genital mycoplasma, the most prevalent pathogen being *G. vaginalis* (33.8%). This falls in line with the conclusion made by Paavonen *et al.*[13] and Shafer *et al.*,[14] who have revealed that genital mycoplasma is detected significantly more often in women with bacterial vaginosis than in those without. These results are higher than those reported by Agbakoba *et al.*[15] who, while working with 168 women, found that 76 (42.5%) were infected with other organisms, which included *C. albicans* (16.7%), *G. vaginalis* (11.9%) and *T. vaginalis* (3.4%). The high prevalence of co-infection of mycoplasma with candidiasis opposes the findings of Koch *et al*.,[16] who reported that mycoplasmas occur less frequently with genital candidiasis. Agbakoba and associates[9] supported Koch and colleagues[16] by saying that mycoplasmas thrive better in near alkaline pH but candida lowers the vaginal pH. The high prevalence of *G. vaginalis* is owing to the fact that it is one of the main causes of bacterial vaginosis.[17] All these isolates may play various interactive roles in the urogenital tract of women, which could lead to various adverse conditions in the long run, hence should not be neglected. The 63.2% colonisation with *C. albicans*, *G. vaginalis* or *T. vaginalis* shows that patients diagnosed with any of these infections have an increased chance of infection with genital mycoplasmas.

Regarding antibiotic susceptibility, pristinamycine was the most sensitive (with 92% sensitivity) and trimetroprine-sulfamethoxazole the most resistant (with 8% sensitivity). The drug of choice between 1983 and 1984 was tetracycline and the alternative drug was erythromycin.[1] The high sensitivity of *U. urealiticum* to erythromycine and doxycycline with resistance to lincomycine was observed by Jafar and colleagues.[18] The latter also observed that *M. hominis* was sensitive to lincomycine and doxycycline, as found in this study. Presently, both *M. hominis* and ureaplasma strains are highly resistant to tetracycline.[18] Ryan *et al*.[19] have revealed that sexually transmitted diseases are common among sex workers in Cameroon. It is therefore important that erythromycin be administered to patients presenting with symptoms of non-chlamydial, non-gonococcal uretrithis, as recommended by Jenkins[20] because, in most areas in developing countries, laboratories facilities are rare. This

Table 3. Antibiotic sensitivity of genital mycoplasmas isolated.

Antibiotics	General sensitivity			UU			MH			UU and MH		
	S	I	R	S	I	R	S	I	R	S	I	R
	n=65			n=41			n=4			n=20		
Lincomycine	7	2	56	2	2	37	3	0	1	2	0	18
Trimethoprim-sulfamethoxazole	5	1	59	2	1	38	0	0	4	3	0	17
Erythromycin	41	1	23	40	1	0	0	0	4	1	10	19
Doxycycline	44	0	21	24	0	17	4	0	0	16	0	4
Pristinamycine	60	1	4	40	1	0	4	0	0	16	0	4
Roxithromycine	31	14	20	29	12	0	0	1	3	2	1	17
Azithromycine	43	2	20	29	12	0	0	1	3	3	0	17
Josamycine	49	4	12	41	0	0	4	0	0	4	4	12
Ciprofloxacin	8	9	48	4	5	31	2	1	1	2	2	16
Ofloxacine	7	48	10	3	34	4	1	2	1	3	12	5

S,sensitive; I, intermediate; R, resistant.

Table 4. Antibiotic sensitivity to genital mycoplasmas isolated.

	PT	JM	DO	AZM	E	ROX	CIP	OFX	L	SXT
MH+UU+coinfection	60	49	44	43	41	32	8	7	7	5
Percentage (%)	92	75	68	66	63	49	12	10	10	8
PT>JM>DO>AZM>E>ROX>CIP>OFX>L>SXT										

PT, pristinamycine; JM, josamycine; DO, oxycycline; AZM, azithromycin; E, erythromycin; ROX, roxithromycin; CIP, ciprofloxacin; OFX, ofloxacine; L, lincomycine; SXT, trimethoprim-sulfamethoxazole.

is confirmed by our results showing that about 63% of the isolates were sensitive to this antibiotic.

Conclusions

Genital mycoplasma is a problem in Cameroon and infected women should be treated together with their partners. The incidence of *U. urealyticum* and *M. hominis* infections of the female genitourinary system is distinctly correlated with age and sexual activity. The diagnosis of the inflammatory states of the genitourinary system and their complications should involve tests for these pathogens, especially for *U. urealyticum*. Pristinamycine was the most effective antibiotic (92%) and sulfamethoxazole the most resistant (8%) antibiotic to genital mycoplasmas.

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