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, pyoepidemic infection, septical abortions, low birth weight, nongonococcal urethritis and postnatal 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 Yaounde 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=0.29-7.8%] 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%). Pristinamycin 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. 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. Genital mycoplasmas have been implicated in pelvic inflammatory diseases, puerperal infection, septical abortions, low birth weight, nongonococcal urethritis and postnatal as well as spontaneous abortion and infertility. 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. Mycoplasma genitalium has become the third most frequent pathogen causing nongonococcal urethritis. It has also been reported that Mycoplasma hominis is a cofactor for bacterial vaginosis and pelvic inflammatory disease. Gdoura et al., in a study conducted in Tunisia, have shown a high prevalence of M. hominis.

Our study investigated the genital mycoplasma species M. hominis and Ureaplasma urealiticum and their drug sensitivity patterns. These microorganisms are cultured as compared to other mycoplasmas, which are fastidious and cannot be cultured but can only be studied using the PCR method. Our study also investigated the relationship between genital mycoplasma infection and Candida albicans, Gardnerella 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.

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Yoaundé 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

Mycoplast Evolution 3 Culture Media

Mycoplast 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. Mycoplast 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...
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 of significance of associations between categorical variables, and the student-t test to test statistical significance of hypotheses for continuous variables used were.

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 mycoplasmas

The mycofast kit for mycoplasma also contained wells for different antibiotics, which included lincomycin, trimethoprim-sulfamethoxazole, erythromycin, doxycycline, pristinamycine, ciprofloxacin, ofloxa- cine, 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 lincomycin and ciprofloxacin. M. hominis was most sensitive to lincomycin, doxycycline, pristinamycine and josamycine and least sensitive to sulfamethoxazole, erythromycin, roxithromycin and azithromycin (Table 3). The most sensitive antibiotic to geni-
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 associates8 supported Koch and colleagues16 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, pristinamycin was the most sensitive (with 92% sensitivity) and trimetoprine-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. urealyticum to erythromycin 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-chlamydiaid, non-gonococcal urethritis, as recommended by Jenkins20 because, in most areas in developing countries, laboratories facilities are rare. This

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>General sensitivity</th>
<th>UU</th>
<th>MH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lincomycin</td>
<td>S=65</td>
<td>I=41</td>
<td>n=4</td>
</tr>
<tr>
<td>Trimethoprin-</td>
<td>S=50</td>
<td>I=59</td>
<td>n=4</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>E=40</td>
<td>I=23</td>
<td>n=4</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0=1</td>
<td>1=0</td>
<td>4=0</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0=1</td>
<td>1=0</td>
<td>4=0</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>0=1</td>
<td>1=0</td>
<td>4=0</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>0=1</td>
<td>1=0</td>
<td>4=0</td>
</tr>
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<td>0=1</td>
<td>1=0</td>
<td>4=0</td>
</tr>
<tr>
<td>Josamycine</td>
<td>0=1</td>
<td>1=0</td>
<td>4=0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0=1</td>
<td>1=0</td>
<td>4=0</td>
</tr>
<tr>
<td>Ofloxacine</td>
<td>0=1</td>
<td>1=0</td>
<td>4=0</td>
</tr>
</tbody>
</table>

Table 4. Antibiotic sensitivity to genital mycoplasmas isolated.

<table>
<thead>
<tr>
<th>MH+UU+co-infection</th>
<th>PT</th>
<th>JM</th>
<th>DO</th>
<th>AZM</th>
<th>E</th>
<th>ROX</th>
<th>CIP</th>
<th>OFX</th>
<th>L</th>
<th>SXT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage (%)</td>
<td>72</td>
<td>75</td>
<td>68</td>
<td>66</td>
<td>63</td>
<td>49</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

PT>JM>DO>AZM>E>ROX>CIP>OFX>L>SXT

PT, pristinamycin; JM, josamycine; DO, doxycycline; AZM, azithromycin; E, erythromycin; ROX, roxithromycin; CIP, ciprofloxacin; OFX, ofloxacine; L, lincomycine; SXT, trimetoprin-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.

References