Prevalence and influence of *Mycoplasma hominis* and *Ureaplasma urealyticum* in 218 African pregnant women and their infants

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Accepted 21 May 1994

Abstract

Objectives: To estimate the prevalence of *Ureaplasma urealyticum* (Uu) and *Mycoplasma hominis* (Mh) in the lower genital tract of pregnant women, their evolution during pregnancy, and the effect of these pathogens on the outcome of pregnancy in Equatorial Africa. Study design: 218 pregnant women were followed from before 20 weeks gestational age through delivery. Samples were taken from the cervix at every visit and from the newborn at delivery and tested for Uu and Mh. The data were analysed using Student's t-test, the Mann-Whitney, or the χ²-test. Results: The prevalence of cervical colonization by *Ureaplasma urealyticum* and *Mycoplasma hominis* in pregnant women was 79% and 41% respectively. Colonization with Uu and Mh increased significantly throughout pregnancy (P < 0.001). Their presence was associated with lower gestational age at delivery, lower birth weight and increased neonatal morbidity and mortality (P < 0.05). Erythromycin therapy did not have any effect on the evolution of Uu and Mh colonization during pregnancy. Conclusion: Uu and Mh are additional factors that might contribute to poor pregnancy outcome in a country where neonatal health is already impaired by many other microorganism.

Keywords: *Ureaplasma urealyticum*; *Mycoplasma hominis*; Pregnancy; Erythromycin; Neonates

1. Introduction

In studies from developed countries, colonization of the endocervix with *Ureaplasma urealyticum* (Uu) and *Mycoplasma hominis* (Mh) has been reported to occur in 50-70% and in 15-30% of pregnant women, respectively [1]. The pathogenicity of *Mycoplasma* species during pregnancy remains controversial [1-6]. Uu and Mh have been implicated in preterm labor and delivery [7-9], low birth weight [5,7], perinatal morbidity and mortality [2,7,10-13], chorioamnionitis [2,3,13] and post partum fever [2,10,13,14]. Materno fetal transmission of Uu and Mh has also been described [13,15,16].

In Africa, where various sexually transmitted diseases have a well known adverse effect on perinatal outcome, few published studies [17,18] have evaluated the prevalence of mycoplasma during pregnancy.

This study was designed to estimate the prevalence of *Ureaplasma urealyticum* (Uu) and *Mycoplasma hominis* (Mh) in the lower genital tract of African pregnant women, their evolution during pregnancy, and their effect on the outcome of pregnancy in Equatorial Africa.

2. Patients and methods

The population of the southern province of Gabon (Equatorial Africa) is 170 000, and 30 000 live in Franceville. This area is a transition zone between the equatorial rain forest and the savannah.

From September 1990 to November 1991, 1840 pregnant women registered at the maternity unit of Franceville hospital. After approval by the local ethics
committee 218 women (representative of the local obstetrical population) with a singleton pregnancy at gestational age (GA) <20 weeks of amenorrhea (WA) were included in the study. Informed consent was obtained for each patient.

Gestational age was determined by the date of the last menstrual period, and by ultrasonic measurement of crown-rump length, or biparietal diameter. Patients were evaluated at each visit prior to 20 GA and again at 25 and 33 GA. The assessment included endocervical and urethral swabs for Uu, Mh, Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Group B streptococcus, and for other microorganisms. Patients were also tested for syphilis (VDRL (Diagnostic Pasteur, Marnes la Coquette, France) and TPHA (Berhing Diagnostics)) and malaria (blood smear stained by Giemsa). HTLV 1 and HIV serostatus were determined by an enzyme linked immunosorbent assay (ELISA) and confirmed by Western blot (Dupont de Nemours, Wilmington, DE).

At delivery, Uu and Mh swabs were taken from the cervix, the fetal side of the placenta and from the newborn (ear and gastric fluid). The neonatal report included gestational age at delivery, birthweight, Apgar score at 1 min, neonatal jaundice, and weight gain during the first week after delivery expressed in percent of birthweight. Specific diseases related to Uu and Mh, such as pneumonia and meningitis [7], were recorded during the first week of life. All the infants were breastfed and kept in the hospital for at least 5 days.

We determined the local cut-off value for low birth weight (LBW) at term (≥37 GA), which was 2500 g (10th percentile) over 1677 deliveries (unpublished data).

*Chlamydia trachomatis* was detected by direct immunofluorescence (Chlamydia direct immunofluorescence bioMérieux 55521, Paris, France) and their presence was confirmed by culture on McCoy cells; infected patients were treated by erythromycin, 3 g daily for 3 weeks. *Neisseria gonorrhoeae* and other microorganisms were cultured on appropriate media after transport on TGV transport medium (Diagnostic Pasteur, Paris, France). Gonococcal infections were treated accordingly. A positive serology for syphilis was treated by benzylpenicillin benzathine 2.4 MU1 intramuscularly twice at a 10-day interval. Each patient treated was given a prescription for her partner to be treated as well.

*Mycoplasma* were screened by MYCOFAST<sup>®</sup> kits (International Mycoplasma, Toulon, France) after transport at 4°C on appropriate liquid media. These kits detect the metabolic activity of mycoplasma (urease (+) for Uu and arginine dehydrogenase (+) for Mh) in selective anaerobic liquid media containing antibiotics and antifungal agents. Identification was carried out after 24 and 48 h of incubation at 37°C by colorimetry (phenol turns from yellow to red in an alkaline medium). The kits rely on Identibiogram<sup>®</sup> (identification by the profile of sensitivity to antibiotics) [19]. The method has a sensitivity of 95% and a specificity of 97% when compared with conventional cultures [20]. Uu was quantified as negative, ≤10<sup>3</sup>, >10<sup>4</sup>, or ≥10<sup>5</sup> units per milliliter of media. Mh was quantified as <10<sup>4</sup> or ≥10<sup>5</sup> unit per milliliter of media. Standard cultures performed simultaneously did not show any growth of urease (+) or arginine dehydrogenase (+) bacteria (i.e. *Proteus vulgaris, Proteus mirabilis*, etc.). Although this method of screening is not classical, it was suitable under our working conditions.

Statistical analysis was made by Student’s *t*-test for variables of normal distribution, and the Mann–Whitney for other variables. The *χ*²-test was used for qualitative variables. Confidence intervals for the odds ratio in 2 × 2 tables were obtained by using Cornfield’s approximation (Epi info<sup>®</sup> software, version 5).

### 3. Results

The mean gestational age at booking was 16.2 weeks (S.D. = 2.8; range, 7.5–20 weeks; n = 218). Thirty patients (13.8%) tested negative for mycoplasma (Uu or Mh); 62 patients (28.7%) presented with Uu and Mh in both endocervix and urethra. Colonization of the endocervix by Uu and by Mh was found in 172 (79%) and 89 (41%) women, respectively, and colonization of the urethra by Uu and by Mh occurred in 82% and 34% of the cases, respectively. There was a strong correlation between colonization of urethra and endocervix by Uu and Mh (*P* < 0.0001) (Table 1). Uu and Mh colonization was not correlated to ethnic origin, total number of sexual partners, or marital status.

There was a positive correlation between colonization of the endocervix by Mh and an urban setting (88.7% of patients with Mh in endocervix lived in Franceville whereas 77.7% of patients free of Mh lived in Franceville) (*P* < 0.05; OR = 2.29; CI<sub>95</sub>, 1.01–5.58); age at first coitus under 15 years (*P* < 0.05; OR = 2.3; CI<sub>95</sub>, 1.3–3.7).

<table>
<thead>
<tr>
<th></th>
<th>Uu Endocervix</th>
<th>Endocervix</th>
<th>Mh Endocervix</th>
<th>Endocervix</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>165</td>
<td>13</td>
<td>69</td>
<td>5</td>
</tr>
<tr>
<td>−</td>
<td>7</td>
<td>33</td>
<td>20</td>
<td>124</td>
</tr>
</tbody>
</table>

*OR = 9.29* *P < 0.0001* OR = 90.79 *P < 0.0001*

*OR*, odds ratio.
CIgs, 0.98–5.67); a positive syphilitic serology (4.5% for endocervix positive for Mh against 0% for endocervix negative for Mh) (P < 0.02); recovery of *Trichomonas vaginalis* from the endocervix (12.3% for Mh-positive against 1.5% for Mh-negative) (P < 0.001; OR = 8.96; CI 95, 1.86–84.47) and of *Chlamydia trachomatis* (24.7% for Mh-positive against 13.1% for Mh-negative) (P < 0.001; OR = 8.96; CI 95, 1.86–84.47). There was a non-significant trend towards an increased rate of cervical colonization with Uu and Mh in older and multiparous women, and women with a history of stillbirth.

There was no correlation between mycoplasma recovery and *Neisseria gonorrhoeae* in the cervix (0.9% of the population), *Plasmodium* in the blood smear (31.2% of the population), HIV- or H11V1-positive serology (respectively 1.8% and 9.1% of the population).

There was a negative correlation between endocervical colonization by Uu and a history of abortion (9% in Uu-negative endocervix versus 2% in Uu-positive endocervix) (P < 0.02; OR = 0.18; CI 95, 0.22–1.05), and recovery of Group B Streptococcus in the cervix (6.5% in Uu-negative endocervix against 1.1% in Uu-positive endocervix) (P < 0.05; OR = 0.17; CI 95, 0.03–1.04).

One hundred and ninety-three out of 218 patients were followed up to delivery and, among them, 181 were available for Uu and Mh screening at delivery. The rate of Uu and Mh endocervix colonization (in these 181 patients) increased significantly (P < 0.001) from the first visit to delivery: 78.4–94.5% and 41.4–58% for Uu and Mh, respectively (Fig. 1). At delivery 98.6% of first visit positive endocervix for Uu remained positive while 20.5% of first visit negative endocervix for Uu remained negative; for Mh the rates were 78.7% and 56.6%, respectively.

There was an inverse relationship between the mean gestational age at delivery (38.6 ± 1.86 GA) and colonization of the endocervix and of the neonate (irrespective of the site of sampling) by Uu and Mh (P < 0.05). The duration of membrane rupture could not be assessed accurately as most of the women arrived in the second stage of labor.

The mode of delivery was not influenced by colonization with mycoplasma. The incidence of stillbirth (Table 3) was 3.64% of all deliveries, and this rate increased with the recovery of Uu in the gastric fluid (P < 0.01). A total of 169 (75%) newborns were followed up to 7 days postnatally. The rate of neonatal colonization (Table 2) by Uu or Mh was 58.3% and 22.2%, respectively. The mean birthweight was 3015.1 ± 480.9 g; there was a decrease in birthweight with colonization of the endocervix by Mh and with colonization of the neonate (irrespective of the site of sampling) by Uu (P < 0.05). Although the differences were not significant, the rate of LBW and prematurity (Tables 2–4) increased with colonization of the endocervix and the newborn (whatever the site) by Uu and Mh. The mean Apgar score at 1 min (Tables 3 and 4) decreased with colonization of the neonate by Uu irrespective of the site of sampling (P < 0.05).

During the first 7 days of life, neonatal jaundice (Tables 3 and 4) occurred in 3.48% of all deliveries. This rate increased with the rate of gastric fluid colonization by Uu (P < 0.05). The weight gain in the first week of life (Tables 3 and 4) was 5.5 ± 5.4, and decreased with the rate of gastric fluid colonization by Uu and irrespective of the site of sampling for Uu and Mh (P < 0.05).

Erythromycin therapy decreased the number of endocervix cultures positive for *Chlamydia trachomatis* from 18.5% to 10.3% (P < 0.05) (Fig. 1) but this therapy showed no effect on the evolution of Uu and Mh colonization in the endocervix from the first visit to delivery, and no effect on pregnancy outcome (Table 5). However, there was a higher weight gain (in the first week of life) in infants born from treated mothers (P < 0.05).
Table 2
Rate of neonatal colonization, low birthweight and preterm delivery related to level of maternal colonization

<table>
<thead>
<tr>
<th>Endocervix colonization</th>
<th>n</th>
<th>Neonatal colonization (%)</th>
<th>LBW (%) (&lt;2500 g)</th>
<th>Birthweight (g) (mean ± S.D.)</th>
<th>Preterm delivery (%) (&lt;37 SA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uu (u/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>38</td>
<td>53.8</td>
<td>2.63</td>
<td>3079 ± 387</td>
<td>5</td>
</tr>
<tr>
<td>≤ 10³</td>
<td>6</td>
<td>50</td>
<td>16.7</td>
<td>3068 ± 403</td>
<td>0</td>
</tr>
<tr>
<td>10⁴</td>
<td>25</td>
<td>37.7</td>
<td>7.7</td>
<td>3068 ± 529</td>
<td>1.14</td>
</tr>
<tr>
<td>≥ 10⁵</td>
<td>112</td>
<td>59.6</td>
<td>8</td>
<td>2978 ± 497</td>
<td>15.1</td>
</tr>
<tr>
<td>Mh (u/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>107</td>
<td>20</td>
<td>4.8</td>
<td>3080 ± 459*</td>
<td>8.8</td>
</tr>
<tr>
<td>≥ 10⁴</td>
<td>74</td>
<td>24</td>
<td>10.4</td>
<td>2921 ± 495*</td>
<td>15.2</td>
</tr>
</tbody>
</table>

Abbreviations: Uu, Ureaplasma urealyticum; Mh, Mycoplasma hominis.
*P < 0.02 (Student’s t-test).

Table 3
Neonatal colonization and neonatal outcome

<table>
<thead>
<tr>
<th>%</th>
<th>Apgar 1 min (mean ± S.D.)</th>
<th>Stillbirth (%)</th>
<th>Jaundice (%)</th>
<th>Weight gain (%) (mean ± S.D.)</th>
<th>LBW (%) (&lt;2500 g)</th>
<th>Birthweight (g) (mean ± S.D.)</th>
<th>Preterm delivery (%) (&lt;37 SA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric fluid Uu (u/ml)</td>
<td>0</td>
<td>63.3 ± 1.4*</td>
<td>0**</td>
<td>0.87***</td>
<td>6.4 ± 3.2*</td>
<td>4.8</td>
<td>3039 ± 480</td>
</tr>
<tr>
<td></td>
<td>≤ 10³</td>
<td>3.3 ± 0.8</td>
<td>0</td>
<td>3.7 ± 4.2</td>
<td>0</td>
<td>0</td>
<td>3032 ± 670</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
<td>12.2 ± 2.2</td>
<td>4.5</td>
<td>13.6***</td>
<td>2.6 ± 4.3</td>
<td>20</td>
<td>2883 ± 481</td>
</tr>
<tr>
<td></td>
<td>≥ 10⁵</td>
<td>21.2 ± 2.2*</td>
<td>10.5**</td>
<td>5.26</td>
<td>3 ± 6*</td>
<td>9.77</td>
<td>2945 ± 502</td>
</tr>
<tr>
<td>Gastric fluid Mh (u/ml)</td>
<td>0</td>
<td>83.3 ± 1.8</td>
<td>2.6</td>
<td>3.3</td>
<td>5.5 ± 5.3</td>
<td>6</td>
<td>3029 ± 482</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
<td>16.7 ± 2</td>
<td>3.3</td>
<td>3.3</td>
<td>3.3 ± 5.8</td>
<td>14.8</td>
<td>2929 ± 539</td>
</tr>
</tbody>
</table>

Abbreviations: Uu, Ureaplasma urealyticum; Mh, Mycoplasma hominis.
Weight gain is expressed as (weight day 7 - birthweight) + birthweight.
*P < 0.02 (Student’s t-test).
**P < 0.01 (x²-test).
***P < 0.05 (x²-test).

Table 4
Neonatal colonization and neonatal outcome

<table>
<thead>
<tr>
<th>%</th>
<th>Apgar 1 min (mean ± S.D.)</th>
<th>Stillbirth (%)</th>
<th>Jaundice (%)</th>
<th>Weight gain (%) (mean ± S.D.)</th>
<th>LBW (%) (&lt;2500 g)</th>
<th>Birthweight (g) (mean ± S.D.)</th>
<th>Preterm delivery (%) (&lt;37 SA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uu</td>
<td>+</td>
<td>58.3 ± 2.3*</td>
<td>4.76</td>
<td>4.76</td>
<td>4.2 ± 5.6*</td>
<td>10.1</td>
<td>2916 ± 497</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>41.7 ± 0.9*</td>
<td>0</td>
<td>1.33</td>
<td>6.4 ± 4.9*</td>
<td>4.2</td>
<td>3148 ± 453</td>
</tr>
<tr>
<td>Mh</td>
<td>+</td>
<td>22.2 ± 2</td>
<td>2.5</td>
<td>2.5</td>
<td>3.4 ± 5.3**</td>
<td>11.4</td>
<td>2896 ± 519</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>77.8 ± 1.8</td>
<td>2.8</td>
<td>3.6</td>
<td>5.7 ± 5.4**</td>
<td>6.4</td>
<td>3046 ± 480</td>
</tr>
</tbody>
</table>

Weight gain is expressed as (weight day 7 - birthweight) + birthweight.
Abbreviations: Uu, Ureaplasma urealyticum; Mh, Mycoplasma hominis. Colonizations defined as positive were cultured from gastric fluid and/or placenta and/or ear.
*P < 0.01 (Student’s t-test).
**P < 0.05 (Student’s t-test).
Weight gain(%) is expressed as (weight day 7 - birthweight) / birthweight.

Table 5

<table>
<thead>
<tr>
<th>Gestational age at delivery (Mean ± S.D.)</th>
<th>Weight (g) (Mean ± S.D.)</th>
<th>Apgar 1 min (Mean ± S.D.)</th>
<th>Weight gain (Mean ± S.D.)</th>
<th>Stillbirth</th>
<th>UU+ (%)</th>
<th>Mh+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERY(-) n = 140</td>
<td>38.3 ± 3</td>
<td>3031 ± 519</td>
<td>8.9 ± 2.3</td>
<td>4 ± 5*</td>
<td>4.3</td>
<td>80</td>
</tr>
<tr>
<td>ERY(+) n = 53</td>
<td>38.7 ± 1.6</td>
<td>2972 ± 368</td>
<td>9.5 ± 1.5</td>
<td>7 ± 6*</td>
<td>1.9</td>
<td>77.3</td>
</tr>
</tbody>
</table>

Weight gain(%) is expressed as (weight day 7 - birthweight) / birthweight.

*P < 0.05 (Student’s t-test).

4. Discussion

The prevalence of Uu and Mh was high when compared with the overall prevalence reported in developed countries, but was similar to rates in black women in the USA [5]. Low genital tract colonization with Mh and Uu increase throughout pregnancy. This may be related to hormonal and immunological modification, as suggested by previous studies [16,21,22]. Iwasaka and coworkers [16,21,22] stated that estrogens increased the colonization of mouse genital tract by Uu and that colonization in human female was linked to their hormonal status; however, such a hormonal influence has not been shown for Mh. At high risk for Mh colonization were women with an early onset of sexual activity, an urban setting, a history of Chlamydia trachomatis and/or Trichomonas vaginalis infection, and a positive syphilitic serology. These findings suggest sexual transmission of Mh [6]. Sexual practice of both partners may be modified during pregnancy; however, this is made unlikely by the fact that Chlamydia trachomatis infection was decreased following a single course of erythromycin.

While Uu colonization was not related to sexual transmission, there was an inverse relationship with a history of abortion. No explanation was found for this observation. More surprising was the negative correlation between Uu and Group B streptococcus. We found the latter occurring at a lower rate when compared with studies in developed countries [23]. Group B streptococcus grows better in slightly acidic medium [24] and could be inhibited by Uu-induced urea transformation and subsequent alcalinisation of the vagina [27].

The rate of neonatal colonization was higher than that previously reported in the literature [16] and depended on the rate of maternal colonization (Table 2). This may be related to more massive colonization with Uu (10^5 u/ml) and/or poor hygiene. While the incidence of low birthweight and preterm delivery (Tables 2-4) were not significantly different in colonized and non-colonized patients, gestational age at delivery and birthweight were inversely correlated to maternal and neonatal colonization with Mh and Uu irrespective of maternal age, Chlamydia trachomatis infection and malaria. In an extensive review of the literature Romero et al. [8] concluded that neither Uu nor Mh were associated with LBW and prematurity, except in women with impaired immune defenses.

Our finding were not adjusted for the possible confounding effects of bacterial vaginosis, which has been shown to be related to preterm delivery [25]. Therefore, it cannot be ruled out that Mh may be associated with preterm delivery through an association with bacterial vaginosis. The association between colonization with Uu and Mh with both fetal distress and stillbirth might be coincidental, since fatal outcomes were related to obstetrical problems (abruptio placenta, shoulder dystocia, circular umbilical cord, macerated fetus). Unfortunately, we could not assess the relationship between colonization by Mycoplasma species and the duration of rupture of the membranes. Babies colonized by Uu or Mh not only had the lowest birthweight but also had the lowest weight gain during the first week of life and the highest rate of neonatal jaundice. These results are suggestive of chronic neonatal infection by Uu and/or Mh, but we did not find any evidence of systemic infection by mycoplasma, such as pulmonary or central nervous system infection as described by Cassel et al. [13].

McCormack et al. [26] showed that women treated in the third trimester with erythromycin gave birth to neonates with mean birth weights significantly greater and a rate of LBW significantly lower than those of placebo-treated women. In our study the weight gain in the first week was higher in neonates from treated mothers, but if erythromycin seemed to be effective on Chlamydia trachomatis infection, this antibiotic regimen did not influence the maternal or neonatal colonization by Uu and Mh. A low diffusion of this drug in the lower genital tract has been reported in previous studies where no effect of erythromycin was reported [4]. In contrast, in vitro sensitivity assays have always demonstrated sensitivity of Uu to erythromycin (when alone in culture).
5. Conclusion

The prevalence of cervical colonization by Uu and Mh increased significantly during pregnancy. There was a correlation with decrease in birthweight and earlier gestational age at delivery, although we could find no significant correlation between maternal and neonatal colonization with Mh and Uu and the incidence of low birthweight or prematurity. We did not find any evidence for a direct pathogenic effect of mycoplasma, but there was an increased neonatal morbidity and mortality in neonates from colonized mothers suggesting that Uu and Mh might be additional factors of poor pregnancy outcome in a country where neonatal outcome is already impaired by poor socioeconomic and medical resources, and by other microorganisms. However, the additional effects of bacterial vaginosis could not be assessed in this study. Our data suggest that screening for mycoplasma species in the first trimester of pregnancy would be of benefit if an effective treatment could be offered. Because results with erythromycin are disappointing, studies evaluating the efficacy of other antibiotics should be carried out. If Mycoplasma species are considered to be only ‘witnesses’ of disturbance of vaginal microflora, then their treatment would not improve perinatal outcome.

Acknowledgments

Financial support was obtained by C.I.R.M.F. (70% funding from Gabonese Government and 30% from ELF Gabon) and by International Mycoplasma who provided us with materials. We are grateful to the C.I.R.M.F and the CHF staff, especially B. de la Fayolle, F. de Beuvron, Y. Martin Prevel, O. Joumas, J. Tissedre, the midwives and Mrs Toure. We are grateful to P.H. Dubreuil from International Mycoplasma for his technical assistance.

References

