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*ORIGINAL ARTICLE*

# Presence of Mycoplasma and Ureaplasma Species in theVagina of Women of Reproductive Age.

## *Présence des espèces Mycoplasmes et Ureaplasmes dans le Vagin des Femmes en Age de Reproduction.*

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**ABSTRACT**

OBJECTIVE: **Against the background of genital mycoplasmas being implicated as pathogens in the genital tracts of adult humans, this study was carried out to determine the prevalence of genital mycoplasmas among women of reproductive age attending a tertiary-care hospital in Nigeria.**

METHODS: **One hundred and sixty-eight high vaginal specimens from 114 pregnant and 54 non-pregnant women with or without complaints were studied for the presence of genital mycoplasmas using selective bacteriological culture techniques. Isolates were identified biochemically and serologically.**

RESULTS: **Sixty (35.7%) isolates of genital mycoplasmas were obtained from the study population. Identification showed 30 (17.9%) as *Mycoplasma* species, 13(7.7%) as *Ureaplasma* species and 17(10.1%) were positive for both organisms. Thirteen (11.4%) ureaplasma isolates were from pregnant women and 17 (31.5%) from non-pregnant women (p< 0.05). Thirty-eight (80.8%) of the *Mycoplasma* species were identified as *Mycoplasma hominis*, 6(12.8%) as *M. fermentans* and 3(6.4%) as *M. penetrans*. High prevalence of the *Mycoplasma* species were obtained from asymptomatic pregnant women (84.8%) while the *Ureaplasma* species were found more from symptomatic non-pregnant women – 35.3% from infertility patients, 29.4% from those with vaginal discharge and 29.4% from those with other gynaecological complaints.**

CONCLUSION: **These findings could be important since genital mycoplasmas have been associated with various adverse conditions especially in pregnant women and neonates while Ureaplasma species have been implicated in infertility. Their specific roles need further investigations. WAJM 2007; 26(1): 28 – 31.**

**Keywords: Genital mycoplasmas, prevalence, vaginal tract, Nigerian women.**

**RESUMÉ**

***Objectif:*** *Contre le contexte des mycoplasme étant impliqués comme étant des pathogenes des appareils génitaux des adultes, cette étude a pour but de déterminer la prévalence des mycoplasmes génitaux chez les femmes en âge de reproduire qui visitent un hôpital de soins tertiaires au Nigéria.*

***Méthodes:*** *Cent soixante-huit prélevement cervicaux vaginaux de 114 femmes enceintes et 54 femmes non encientes avec et sans symptomes ont été étudiés pour chercher la présence des mycoplasmes génitaux en utilisant les techniques de cultures bactériologiques sélectives. Les souches ont été biochimiquement et sérologiquement identifiées.*

***Résultats:*** *Soixante (35.7%) souches des mycoplasmes génitales ont été obtenues dans la population en etude. 30 (17.9%) souches de Mycoplasmes et 13(7.7%) espèces Ureaplasmes ont été identifiées, et 17(10.1%) specimens avaient les deux organismes. Treize (11.4%) souches d'Ureaplasmes provenaient des femmes enceintes et 17 (31.5%) des femmes qui n'étaient pas enceintes. (p< 0.05). Trente huit (80.8%) des espèces Mycoplasmes ont ete identifiées comme étant des Mycoplasma hominis, 6(12.8%) comme M. fermentans et 3(6.4%) comme étant M. penetrans. Une prevalence élevée des espèces Mycoplasmes ont été obtenues des femmes enceintes asymptomatiques (84.8%) alors que les espèces Ureaplasmes ont été isolées chez des femmes symptomatiques qui n' étaient pas enceintes – 35.3% chey des patients qui sont stériles, 29.4% de celles qui ont des sécrétions vaginales et 29.4% de celles avec d'autres problemes gynécologiques.*

***Conclusion:*** *Ces résultats peuvent être d'une grande importance puisque les mycoplasmes ont été associées à plusieurs conditions défavorables plus particulièrement chez les femmes enceintes et nouveaux nés tandis que les espèces Ureaplasmes ont été impliquées dans l'infertilité. Leur role dans l;infertilite necessite beaucoup plus de recherche. **WAJM 2007; 26(1): 28 – 31..***

***Mots Clés: Mycoplasmes génitales, prévalence, paroi vaginal, Femmes Nigérianes.***

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## INTRODUCTION

Members of the Class Mollicutes (generally referred to as "Mycoplasmas") are the simplest and smallest free-living organisms and are unique among prokaryotes in that they lack a cell wall.[1] One of the families in this class - Mycoplasmataceae, comprises 2 genera – the Mycoplasma and the Ureaplasma - both of which are pathogens of humans and animals. In humans both genera are common inhabitants of the oro-pharyngeal and genital mucous membranes and occasionally other sites as well[1]. The genital mycoplasmas are important pathogens of the urogenital tracts of humans and in women they have been reported to be associated with a variety of diseases and conditions like pelvic inflammatory diseases,[2] bacterial vaginosis,[3] vaginitis,[4,5] infertility,[6] poor pregnancy outcomes,[7] and the risk of miscarriage.[8] They have also been isolated from neonates especially the offspring of colonized mothers[9] and *Ureaplasma urealyticum* has been implicated in neonatal respiratory[10] and central nervous system infections[11]. Routine isolation of these organisms has been neglected probably due to the expensive and time-consuming nature of its isolation techniques[12]. Consequent upon these reports, this investigation was carried out to determine the prevalence of genital mycoplasmas (*Mycoplasma* and *Ureaplasma* species) in an unselected sample of pregnant and non-pregnant women from our community.

## SUBJECTS, MATERIALS AND METHODS

Two high vaginal swabs were collected from each of 168 female patients (114 pregnant and 54 non-pregnant) attending both Antenatal and Gynaecology clinics of the University College Hospital, Ibadan, Nigeria and cultured for the presence of genital mycoplasmas. Ninety-seven of the pregnant women were normal antenatal-clinic attendees without complaints while the remaining 17 had complaints of vaginal discharge. The non-pregnant women were Gynaecology clinic patients and of the 54 women, 22 had complaints of infertility, 12 complained of vaginal discharge, six had other complaints while the remaining four had no complaints.

The cultivation medium was the modified Hayflick medium,[13] which in this present study was further modified for the mycoplasma isolates by the non-inclusion of the substrates; glucose and arginine. This was to minimize overgrowth of commensal organisms that might have been present in the vaginal samples. For the *Ureaplasma* isolates, the medium[13] was further modified by incorporating 5ml of 20% urea into every 100ml of medium. The liquid-to-solid culture technique[12] was used in the isolation of the organisms hence the specimens were first inoculated into liquid medium and subcultures made onto corresponding solid medium.

One of the vaginal swabs was inoculated into a mycoplasma broth and the second into ureaplasma broth. All the media (broth and agar) were incubated at 37°C but while the broth media were incubated in air, the solid media were incubated in a candle jar for increased carbon dioxide atmosphere. Incubation was for 24 – 48 hours for ureaplasma media and up to 10 days for Mycoplasma media. Suspected colonies were seen with "fried-egg" appearance when examined with a dissecting microscope. The Mycoplasma isolates were identified using biochemical and serological tests (Agbekoba et al, unpublished data) *Ureaplasma* isolates were identified by their urease activities and were regarded as *Ureaplasma* spp.

The collected data are presented in tables using comparative percentages and the Chi-square ($x^2$) test used for significance.

## RESULTS

Table 1 shows that a total of 47 (28.0%) isolates of mycoplasmas and 30 (17.9%) isolates of ureaplasmas were obtained from the population studied. The breakdown showed 30 (17.9%) as *Mycoplasma* species, 13 (7.7%) as *Ureaplasma* species and 17 (10.1%) were positive for both organisms. Subject-wise, a total of 33 (28.9%) mycoplasmas and 13 (11.4%) ureaplasmas were isolated from pregnant women, as against the 14 (25.9%) mycoplasmas and 17 (31.5%) ureaplasmas from non-pregnant women.

Table 2 shows the distribution of the characterized *Mycoplasma* species by source of specimens. Of the 38 *M.hominis* isolated, 29(76.3%) were from pregnant women and 9(23.7%) from non-pregnant women. Six *M. fermentans* isolates were identified and 3 each (50.0%) were from the pregnant and non-pregnant women respectively. The distribution of the 3 *M. penetrans* isolates showed that 2(66.7%) isolates were from non-pregnant women while 1(33.3%) was from a pregnant woman. However for ureaplasmas, a significant result was obtained whereby 13(11.4%) isolates were from pregnant women and 17 (31.5%) from non-pregnant women ($x^2 = 10.923$; $p < 0.012$).

The distribution of the characterized *Mycoplasma* species according to the complaints of the patients is shown in Table 3. Of the 29 *M. hominis* isolated from the pregnant women 24(82.8%) were from asymptomatic women, 4(13.8%) from those with vaginal discharge and 1(3.4%) from a woman with vaginal bleeding (other complaint). Nine *M. hominis* isolates were from the non-pregnant women, 4(44.4%) from women with infertility problems, and 5(55.5%) from women with vaginal discharge. Of the three *M. penetrans* isolated, two (66.7%) were from non-pregnant women - one with vaginal

**Table 1: Distribution of Isolated Organisms from High Vaginal Swabs by Pregnancy**

| Genital Mycoplasma | Number (%) | | |
|---|---|---|---|
| | Pregnant N=114 | Non-pregnant N=54 | Total N=168 |
| Mycoplasma spp | 25(21.9) | 5( 9.3) | 30(17.9) |
| Ureapasma spp | 5 ( 4.4) | 8(14.8) | 13 ( 7.7) |
| Both | 8 ( 7.0) | 9(16.6) | 17(10.1) |

**Table 2: Distribution of Genital Mycoplasma Species by Pregnancy Status.**

| Mycoplasma spp | Number (%) | | |
|---|---|---|---|
| | Pregnant | Non-pregnant | Total |
| M. hominis | 29(76.3) | 9(23.7) | 38(100) |
| M. fermentans | 3(50.0) | 3(50.0) | 6(100) |
| M. penetrans | 1(33.3) | 2(66.7) | 3(100) |
| Ureaplasma spp | 17(31.5) | 13(11.4) | 30(100) |

discharge and the other asymptomatic; while one (33.3%) was from an asymptomatic pregnant woman. Six isolates were characterized as *M. fermentans*, three from asymptomatic pregnant women and the other 3 from non-pregnant women – two with complaint of infertility and one with vaginal discharge.

**Table 3: Distribution of the Characterized *Mycoplasma* and *Ureaplasma* Spp. According to Symptoms.**

| Organism | Pregnant | Complaint (No. (%) | | | | Total |
|---|---|---|---|---|---|---|
| | | Infertility | Vaginal discharge | Other | Nil | |
| *M. hominis* | Yes | 0 (0) | 4(13.8) | 1(3.4) | 24(82.8) | 29 |
| | No | 4(44.4) | 5(55.5) | 0 (0) | 0 (0.0) | 9 |
| *M. fermentans* | Yes | 0 (0) | 0 (0) | 0 (0) | 3 (100.0) | 3 |
| | No | 2(66.6) | 1(33.3) | 0 (0) | 0 (0) | 3 |
| *M. penetrans* | Yes | 0 (0) | 0 (0) | 0 (0) | 1(100.0) | 1 |
| | No | 0 (0) | 1(50.0) | 0 (0) | 1 (50.0) | 2 |
| Ureaplasma Spp. | Yes | 0 (0) | 1(7.7) | 1 (7.7) | 11 (84.6) | 13 |
| | No | 6 (35.3) | 5 (29.4) | 5 (29.4) | 1 (5.9) | 17 |

## DISCUSSION

Human ureaplasma, formerly know as *U. urealyticum*, now has two species – *U. urealyticum* and *U. parvum*[14] which are only separable by molecular techniques such as polymerase chain reaction (PCR)[1]. However, in this study the Ureaplasma isolates were not separated but were regarded simply as *Ureaplasma* spp. Waites[15] reported that for clinical purposes, separating infections caused by the two species of *Ureaplasma* is not possible or necessary and that they are considered clinically and in the diagnostic laboratory as *Ureaplasma* species.

In this investigation, it was observed that an overall prevalence rate of 35.7% was obtained for the organisms. A breakdown of this result (Table 1) showed that the obtained values were lower than the findings of authors from other parts of the world; de Moreno *et al*[16] reported 39% for *M. hominis*, 13% for *U. urealyticum* and 48% for both organisms. Other reports include Faye-Kette *et al*[17] with an overall prevalence of 73% (20% for *M. hominis*, 22% for *U. urealyticum* and 31% for both organism) and Domingues *et al*[18], who reported a 31.5% and 27.8% for *M. hominis* and *U. urealyticum* respectively. These varying isolation rates may be due to the sensitivities of the isolation media, the cultural techniques and the categories of women used.

A breakdown of the result into the two groups of women (pregnant and non-pregnant) showed a non-significant result for the prevalence rates of *Mycoplasma* species. However the reverse was the case with the *Ureaplasma* species where the prevalence rate obtained for the non-pregnant women was significantly higher than that of pregnant women ($p < 0.05$). Investigations carried out by several others showed that varying recovery rates of these organisms have been obtained from these two groups of women. Values of 72% and 47% for pregnant and non-pregnant women respectively for mycoplasmas and 13% and 21% for pregnant and non-pregnant women respectively for ureaplasmas have been reported.[19]

Three species of mycoplasma were isolated from this study, viz *Mycoplasma hominis, M. fermentans* and *M. penetrans*. *M. hominis* is the predominant mycoplasma species and some of them were from women with vaginal discharge. This organism has been reported as being part of the causative agents of bacterial vaginosis in association with *Gardnerella vaginalis*, *Mobiluncus* species and other anaerobes[3]. Few *Gardnerella vaginalis* were found in this study (author's data) and were in association with *M. hominis* in the pregnant women with vaginal discharge and with both organisms in non-pregnant women (some with vaginal discharge and others with infertility problems). This result therefore shows that *M. hominis* and *Ureaplasma* species could play roles in bacterial vaginosis. *M. hominis* was reported as the only mycoplasma detected significantly more often in women with, rather than in those without, bacterial vaginosis.[20] This organism can also indicate the presence of pelvic inflammatory disease in non-pregnant women[2]. All these conditions could predispose to infertility in the non-pregnant women if ignored.

The *M. fermentans* from the pregnant women were all from asymptomatic patients while those from the non-pregnant women were more from infertile patients. *M. fermentans* was among the earliest genital mycoplasmas isolated.[21] The *M. penetrans* too were more from asymptomatic women. This organism has been reported as one of the emergent mycoplasma species and has been isolated from HIV positive patients[22]. It has also been reported as potential cofactor in Acquired Immune Deficiency Syndrome (AIDS). [23] *M. fermentans* too has recently been reported to play roles, in addition to *M. penetrans*, in the development of AIDS.[22] The role of these species of mycoplasmas in these women is not certain and needs further investigation.

The genital mycoplasmas (*Mycoplasma* and *Ureaplasma*) from pregnant women in this study were observed to be more predominant from the asymptomatic women than women with complaints (Table 3). Other workers have also reported similar findings[24]. This could largely be due to the fact that these pregnant women are apparently normal antenatal clinic attendees with little or no complaints. Also the report that genital mycoplasmas are part of the normal vaginal flora of some women[25] could also have contributed to the high prevalence rate obtained from these asymptomatic women. The pregnant women with complaints of vaginal discharge were quite few and this could have accounted for the low isolation rate from them. On the contrary, genital mycoplasmas from the non-pregnant women were observed to be more from patients with various complaints than those with no complaints. In this present study the non-pregnant women were mostly women with infertility

problems and the result obtained from them in this investigation may suggest a contributory role for genital mycoplasmas especially *Ureaplasma* spp. in female infertility. *Ureaplasma urealyticum* has been implicated in infertility having been recovered more from the endometrial specimens of infertile women than from fertile ones.[6]

The isolation of these organisms in pregnant women should not be treated with levity because both *M. hominis* and *U. urealyticum* have been reported to be the causes of several poor pregnancy outcomes[6-8] and neonatal infections[9-11]. Though most of these women were asymptomatic, Driscoll et al[26] postulated that sub clinical mycoplasma infection is an important cause of reproductive failure. The infected pregnant women were not followed-up to see the outcomes of their pregnancies; nonetheless, in view of the fact that previous existing facts infer likely adverse outcomes, treatment of all such women is hereby suggested to avoid any mishap either to mother or to unborn baby. It is also being suggested that the screening of pregnant women for *Ureaplasma* and *Mycoplasma* species be included as part of routine tests done for each pregnant woman during antenatal. In conclusion, *Ureaplasma* and *Mycoplasma* species are additional bacteria that might contribute to urogenital infections, hence their presence in the urogenital tracts of adult females, albeit asymptomatic, should be treated.

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