Isolation, Characterization and Antibiotic Susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* from Infertile Women in Lagos, Nigeria

ABSTRACT

Background:

Mycoplasma hominis and *Ureaplasma urealyticum* are important aetiologic agents of cervicitis, vaginitis, postpartum sepsis, reproductive infections and infertility.

Patients and Methods:

A total of 90 High vaginal(HVS) and first void urine specimens were collected from infertile women attendees of two fertility clinics while 45 HVS and first void urine specimens were also collected from pregnant women attending antenatal clinics at Lagos University Teaching Hospital and 68 Nigerian Army Reference Hospital(68 NARHY) Lagos. All the specimens were inoculated into *Mycoplasma* broth and subsequently Blood Agar plates, incubated appropriately and identified. Antibiotic susceptibility tests were carried out on the 52 isolates. Polymerase chain reaction (PCR) was used to detect the organisms in all the collected specimens.

Results:

Of the 90 HVS specimens collected from infertile women, 9 (10.0%) were positive for *M. hominis*, while 21 (23.3%) were positive for *U. urealyticum*. For the pregnant women using HVS specimens, 6 (13.3%) were positive for *M. hominis* while 5 (11.1%) were positive for *U. urealyticum*. The first void urine specimens gave lower values in both the infertile and pregnant women. Prevalence of U. *urealyticum* was higher in infertile women than in pregnant women ($p<0.05$). The PCR technique gave higher values of 78.5% and 71.1% using HVS specimens for the infertile and pregnant women respectively for *Mycoplasma/Urealyticum* species. The antibiotic susceptibility test showed that all the isolates of *M. hominis* (n=18) were sensitive to Tetracycline and Ciprofloxacin while all the isolates of *U. urealyticum* (n=34) were sensitive to Tetracycline and Erythromycin.

Conclusion:

The significantly higher prevalence of *U. urealyticum* infection in infertile women compared to the lower prevalence in pregnant women may suggest that *U. urealyticum* can be incriminated in infertility. HVS specimen is preferred over urine specimens for the detection of *Mycoplasma and Ureaplasma.* Application of the PCR method, where affordable, is recommended for rapid and sensitive detection of *Mycoplasma and Ureaplasma* in HVS specimens. Tetracycline is the antibiotic of choice, unless contraindicated, for the treatment of the infections.

Keywords: Prevalence, Infertility, Pregnancy, Nigeria, *Mycoplasma hominis, Ureaplasma urealyticum*.

INTRODUCTION

Infertility is considered when couples have been trying to achieve pregnancy with frequent sexual intercourse for at least a year without success[1]. Documented data revealed that approximately 72.4 million couples are infertile[1]. The causes of 25% of the cases of infertility are still unknown[2]. Majority of infertile females have inflammatory changes of the oviduct or the surrounding peritoneum and most of these alterations are caused by infections[3].

Generally, *Mycoplasma hominis and Ureaplasma urealyticum* have been isolated from genital mucosal surfaces, vagina and cervical parts of females [4, 5]. They have been isolated from genital infections in both males and females[6]. *U. urealyticum* is a major cause of non-chlamydial and non-gonococcal urethritis, chorioamnionitis, acute prostatitis, vaginitis, cervicitis, preterm delivery and sepsis [6, 7]. *M. hominis* is often associated with vaginitis, cervicitis, postpartum sepsis, pyelonephritis, preterm labour and premature birth[8, 9]. Bacterial vaginosis is strongly implicated in female infertility and screening and treatment of bacterial vaginosis during the course of infertility management increased the rate of pregnancy[10]. The isolation of these organisms in the diagnostic laboratories is cumbersome and takes several days to achieve. The identification from clinical specimens using Nucleic Acid Amplification Test is very expensive for routine purposes. We therefore decided to determine the prevalence of *Mycoplasma* infections among infertile and pregnant women in Lagos Metropolis, Nigeria; and to determine the susceptibility patterns of the isolates to some of the commonly prescribed antibiotics.

PATIENTS AND METHODS

Ethical Issues: The study was approved by the Ethical Committees of Lagos University Teaching Hospital (LUTH) and the 68 Nigerian Army Reference Hospital, Yaba (68NARHY). All the specimens (Urine and HVS) were from married infertile women attending the Gynecology Clinics as part of a work-up for fertility investigations after failing to conceive for at least one year of unprotected sexual intercourse. Also included in the study were expectant mothers who were normal antenatal clinic attendees for routine medical attention. Subjects with any clinical symptom of sexually transmitted disease were excluded from the study.

Sample Collection: From July 2012 to September 2012, a total of 270 HVS and urine specimens were collected from the study centres in females aged between 22 to 45 years. One hundred and forty (140) HVS/Urine specimens were collected from infertile women in LUTH and 130 HVS/Urine specimens were from infertile women in 68 NARHY. Ninety specimens (HVS/Urine) were also collected from pregnant women from both study centres. The HVS specimens were collected by clinicians using disposable speculum while the subjects were informed on how to collect the urine specimens devoid of contamination.

***Mycoplasma hominis and Ureaplasma urealyticum* isolation and identification:**

The specimens were inoculated into the *Mycoplasma* transport/growth medium without delay. Clearly labelled specimens were treated by inoculating 0.1ml into 5ml of prepared *Mycoplasma* broth at the site of specimen collection and transported to the laboratory. The remaining urine specimens (about 10ml

each) were stored at -70°C. Both the urine and HVS specimens were incubated for up to 48h at 37°C in 5% CO_2 for *Ureaplasma* and up to 5 days for Mycoplasmas. These were examined daily for turbidity as evidence of growth. Subsequently, the broth cultures were subcultured onto solid blood agar media and incubated at 37°C in 5%CO_2 for 24 to 48 hours for *Ureaplasma* and up to 5 days for Mycoplasmas. Urea for urease activities and L-arginine for arginine utilization were used for biochemical tests. Strains of *M. hominis* ATCC 23114 and *U. urealyticum* ATCC 33175 were used as positive controls. Colonies presenting a fried egg appearance suggested the presence of *M. hominis*, while colonies that were brown and tiny indicated the presence of *U. urealyticum. U. urealyticum* also hydrolysed urea while *M. hominis* metabolised the L. arginine.

Polymerase Chain Reaction Confirmation of *U. urealyticum* and *M. hominis*

A PCR technique was used to detect *U. urealyticum* and *M. hominis*. Two millilitres of urine and HVS broth specimens was used Ref: standard strains of *U. urealyticum* (ATCC 33175); *M. hominis* (ATCC 23114) and *Clostridium difficile* (ATCC BA-2155) were used.

Antimicrobial Susceptibility Test on the Isolates

Antimicrobial susceptibility test was carried out on the isolates using the modified Kirby-Bauer method with Tetracycline (30µg), Gentamycin (10µg), Erythromycin (15µg), Streptomycin (30µg) and Ciprofloxacin (5µg).

RESULTS

A total of 90 High Vaginal Swabs from infertile women and 45 HVS specimens from pregnant women were analysed using culture methods and PCR for *M. hominis* and *U. urealyticum*. Out of the 90 HVS specimens from the infertile women, 9 (10.0%) were positive for *M. hominis* while 21 (23.3%) were positive for *U. urealyticum*. The urine specimens from both the infertile and pregnant women gave lower prevalences of 3.3% for *M. hominis*, 6.7% for *U. urealyticum* and 0.0% for *M. hominis*, 4.4% for *U. urealyticum* respectively (Table 1). HVS specimens yielded more isolates than the urine specimens.

X^2= 72.174; P<0.05.

Table 2 shows the distribution of *Mycoplasma/Ureaplasma* organisms in infertile and pregnant women in the study area using *Ureaplasma* using PCR. Out of the 45 HVS specimens from the pregnant women, 32 (71.1%) were *Mycoplasma/Ureaplasma*. The 78 analysed urine specimens from the infertile women gave a prevalence of 58.9% of *Mycoplasma/Ureaplasma* infections. For the pregnant women, out of the screened 45 urine specimens 12 (26.6%) were positive for *Mycoplasma/Ureaplasma*.

The antibiotic susceptibility patterns of the *M. hominis* and *U. urealyticum* is shown in Table 3. *M. hominis* showed 100% sensitivity to Tetracycline and Ciprofloxacin while *U. urealyticum* showed 100% sensitivity to Tetracycline and Erythromycin.

Table 1: Distribution of *M. hominis* and *U. urealyticum* in Infertile (n=90) and Pregnant (n=45) Women in Lagos, Nigeria.

Source	Organism	No. of Isolates	Percentage
Infertile Women			
HVS	*M. hominis*	9	10.0
	U. urealyticum	21	23.3
Urine	*M. hominis*	3	3.30
	U. urealyticum	6	6.70
Pregnant Women			
HVS	*M. hominis*	6	13.30
	U. urealyticum	5	11.10
Urine	*M. hominis*	0	0.00
	U. urealyticum	2	4.40

Table 2: Distribution of *Mycoplasma/Ureaplasma* Species in Infertile and Pregnant Women in Lagos, Nigeria using PCR

Source	Organism	No. of Isolates	Percentage
Infertile Women			
HVS (n=65)	*Mycoplasma/Ureaplasma*	51	78.5
Urine (n=78)	*Mycoplasma/Ureaplasma*	46	58.9
Pregnant Women			
HVS (n=45)	*Mycoplasma/Ureaplasma*	32	71.1
Urine (n=45)	*Mycoplasma/Ureaplasma*	12	26.6

Table 3: Antimicrobial Susceptibility Patterns of *M. hominis* and *U. urealyticum* Isolated from Infertile and Pregnant Women in Lagos, Nigeria.

Source	Organism	No. of Isolates	Antimicrobial Agent Activities % Sensitive				
			Te	CN	E	CPX	S
HVS	*M. hominis*	15	15(100)	0(0.0)	0(0.0)	15(100)	0(0.0)
	U. urealyticum	26	26(100)	0(0.0)	26(100)	0(0.0)	0(0.0)
Urine	*M. hominis*	3	3(100)	0(0.0)	0(0.0)	3(100)	0(0.0)
	U. urealyticum	8	8(100)	0(0.0)	8(100)	0(0.0)	0(0.0)
Total		52	52(100)	0(0.0)	34(65.4)	18(34.6)	0(0.0)

Key:

Te = Tetracycline (30µg)

CN=Gentamycin (10µg)

E=Erythromycin (15µg)

CPX=Ciprofloxacin (5µg)

S=Streptomycin (30µg)

DISCUSSION

The results of our study showed that *M. hominis* and *U. urealyticum* infections are prevalent among the infertile and pregnant women in Lagos Metropolis, Nigeria. In this study both the HVS and Urine specimens were examined using culture and PCR techniques. The culture technique gave a prevalence, using the HVS specimens, of *M. hominis* (10.0%) and 13.3% for infertile and pregnant women respectively. The urine specimens yielded lower values for *M. hominis* with a prevalence of 3.3% and 0.0% for infertile and pregnant women respectively. A prevalence of 6.7% for infertile women and 4.4% for pregnant women were recorded for *U. urealyticum* infection. The PCR technique expectedly gave higher prevalence of 78.5% and 71.1% using HVS specimens for *Mycoplasma/Ureaplasma* in infertile and pregnant women respectively. The urine specimens gave lower values of 58.9% and 26.6% for *Mycoplasma/Ureaplasma* infection in infertile and pregnant women respectively. The urine specimens gave lower values of 58.9% and 26.6% for *Mycoplasma/Ureaplasma* infection in infertile and pregnant women respectively. One possible explanation for the higher prevalences of these infections in infertile women is hormonal disorders which can lead to reduced levels of immunity and increased bacterial colonization ad survival in the vaginal epithelium[12].

In addition to the considerably high prevalence of *M. hominis* and *U. urealyticum* infections in our study, several published report revealed various prevalence rates. Agbakoba et al[12] reported a prevalence of 35.7% in women and of reproductive age in Ibadan. The prevalence rate of *M. hominis* and *U. urealyticum* in HVS specimens ranged from zero to 3.8%[14, 15]. The prevalence of *U. urealyticum* infection was reported from 20% in South Africa to 41.9% and 51.5% in Italy and Africa respectively[14]. In contrast, the prevalence was lower in some other studies[16, 17]. The varied prevalence rates all over the world may be due to the use of only culture methods or PCR. In addition there are many factors that will affect the culture results such as the use of transport medium, the duration between the collection of the sample and the inoculation, and the duration of incubation. The Nucleic Acid Amplification tests gave higher results due to its sensitivity and it is also a rapid test, although it is more expensive.

In our study, it was observed that *M. hominis* and *U. urealyticum* were detected more from HVS than urine specimens. This is I agreement with Taylor-Robinson[17] who reported that the numbers of isolates from urine specimens were usually 10 fold less than in swabs.

In vitro antibiotic susceptibility test showed that all the isolates of *M. hominis* and *U. urealyticum* were sensitive to Tetracycline. The implication of this finding is that where it is not possible to detect the causative agent of non-gonococccal urethritis, it is safer to prescribe Tetracycline instead

of Erythromycin as being practiced in some centres. *U. urealyticum* isolates in addition to Tetracycline were all sensitive to Erythromycin, while all *M. hominis* isolates were also sensitive to Ciprofloxacin.

In conclusion therefore, we have indicated that *M. hominis* and *U. urealyticum* infections are prevalent in both the infertile and pregnant women in Lagos metropolis. Efforts, where feasible, should be made to detect the presence of these organisms during the work-up of cases of infertility and in pregnancy to prevent the adverse outcomes. We therefore recommend the precise monitoring of fertile and infertile females for the presence of *M. hominis* and *U. urealyticum* and treatment of positive cases to prevent diseases and possibly infertility.

REFERENCES

1. Boivin, J; Bunting L; Collins JA et al. International estimates of infertility prevalence and treatment-seeking: Potential need and demand for infertility medical care. Hum Reprod. 2007;22(16): 1506-1512. Doi:10.1093/hum re/dem046. [PubMed][Cross Ref]

2. Daar AS, Merali Z. Infertility and social suffering: the case of ART in developing countries. In: Vayena E, Rowe P, Griffin D, editors. Report of a meeting on “Medical, ethical and social aspects of assisted reproduction.” Geneva: WHO, 2001. Pp.16-21.

3. Wiesenfeld HC, Hillier SL, Meyn LA et al. Subclinical pelvic inflammatory disease and infertility. Obstet Gynecol. 2012;120(1):37-43. Doi:10.1097/AOG.Ob 013e31825a6bc9. [PubMed][Cross Ref]

4. Capoccia R, Greub G, Baud D. Ureaplasma urealyticum, Mycoplasma hominis and adverse pregnancy outcomes. Curr Opin Infect Dis. 2013; 26(3):231-240.doi:10. 1097/QCO. Ob013e328360db58. [PubMed][Cross Ref]

5. Wang QY, Li RH, Zheng LQ et al. Prevalence and antimicrobial susceptibility of Ureaplasma urealyticum and Mycoplasma hominis in female outpatients, 2009-2013. J Microbiol, Immunol Infection. 2009-2013. [PubMed].

6. Al-Sweih NA, Al-Fadli AH, Omu AE et al. Prevalence of Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma genitalium and Ureaplasma urealyticum infections and seminal quality in infertile and fertile men in Kuwait. J Androl.2012;33(6):1323-1329.doi:10. 2164/jandrol. 111.013821. [PubMed] [Cross Ref].

7. Zhu C, Liu J, Ling Y et al. Prevalence and antimicrobial susceptibility of Ureaplasma urealyticum and Mycoplasma hominis in Chinese women with genital infectious diseases. Indian J Dermatol, Venereology, Leprology. 2012;78(3):406-407 [PubMed].

8. Aydin Y, Atis A, Ocer F et al. Association of cervical infection of Chlamydia trachomatis, Ureaplasma urealyticum and Mycoplasma hominis with peritoneum colonization in pregnancy. J Obstet Gynecol. 2010, 30(8):809-812.doi:10.3109/01443615.2010. 519063. [PubMed][Cross Ref]

9. Campos GB, Lobao TN, Selis NN et al. Prevalence of Mycoplasma genitalium and Mycoplasma hominis in urogenital tract of Brazilian Women. BMC Infect Dis 2015;15(1):60. [PMC free article][PubMed]

10. Salah RM, Allam AM, Magdy AM et al. Bacterial vaginosis and infertility: cause or association? Eur J. Obstet Gynecol Reprod Biol. 2013;167(1): 59-63.doi:10.1016/j. ejogrb. 2012.10.031. [Pub Med] [Cross Ref]

11. Clinical and Laboratory Standards Institute: Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria, approved guideline M45-A Wayne, Pa, 2005, CLSI.

12. Kornats'ka AH. Local humoral immunity in women with combined forms of infertility. Lik sprava. 1998(4): 82-84 [PubMed].

13. Agbakoba NR, Adetosoye AI, Adewole IF. Presence of Mycoplasma and Ureaplasma species in the vagina of women of reproductive age. West Afr J Med. 26(1).

14. Leli C, Mencacci A, Bombaci JC et al. Prevalence and antimicrobial susceptibility of Ureaplasma urealyticum and Mycoplasma hominis in population of Italian and immigrant outpatients. Infez Med 2012; 20(2):82-87 [PubMed]

15. Elias M, Grzesko J, Siejkowski R et al. The presence of Mycoplasma hominis and Ureaplasma urealyticum in the cervical of uterus. Ginekol Pol. 2005; 76(1):28-32. [PubMed].

16. Blanchard A, Yanez A, Dybvig K et al. Evaluation of intraspecies genetic variation within 16S rRNA gene of Mycoplasma hominis and detection by polymerase chain reaction. J Clin Microbiol. 1993;31(5):1358-1361.

17. Taylor-Robinson, D. Genital Mycoplasma infection. Clin Lab Med 9:501-523.