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Mycoplasma genitalium in Infertile and Pregnant Women in Lagos, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The aim of this study is to determine the prevalence of *Mycoplasma genitalium* in infertile and pregnant women in Lagos metropolis, Nigeria.
Study Design: The study is a cross sectional survey for the presence of *M. genitalium* in infertile and pregnant women aged 22 to 45 years that attended the Gynecology and Antenatal clinics at Lagos.
Place and Duration of Study: The study was conducted over a 3 month period within Lagos, Nigeria.
Methodology: First- void urine and high vaginal swab samples were collected from 143 infertile women; while 90 pregnant women had their samples collected from the two hospitals. The samples were immediately inoculated into 5ml of sterile *Mycoplasma* broth and transported to the laboratory. The nucleic acid amplification test (Polymerase Chain Reaction) was used to analyze the samples.
Results: Out of the 143 infertile women, 5 (7.7%) yielded *M. genitalium* while 3 of the 90 pregnant women were positive for *M. genitalium* using the high vaginal swab specimens. The first voided

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urine samples from infertile women gave a percentage of 2.6% (2 positives) while 2 of the urine samples from the pregnant women were positive giving a percentage of 4.4.
Conclusion: The study shows that *M. genitalium* can be demonstrated in both pregnant and infertile women using PCR since it is a very fastidious organism. This pathogen should be routinely sought for in all cases of infertility where no other known case is implicated and in pregnancy to prevent recurrent pregnancy outcome.

Keywords: Mycoplasma genitalium; infertility; pregnancy; Nigeria.

1. INTRODUCTION

Mycoplasma genitalium is a member of genital mycoplasmas, which is emerging as an important etiologic agent of sexually transmitted diseases in humans [1]. This organism is fastidious and so isolation in artificial culture media is difficult and often requires several days and weeks for each isolate to grow [1]. Hence, nucleic acid amplification tests (NAATs) are the better diagnostic tools for *M. genitalium* infections at present because of the speed and specificity.

In women, *M. genitalium* can be detected in the genital tract and is found most commonly in those with genital tract symptoms or those with infected male partners. The presence of *M. genitalium* is associated with cervicitis and urethritis in women [2,3] and the inoculation of the organism in non-human primates leads to both lower genital tract disease and salpingitis [4]. *M. genitalium* has been detected in the endometrium of women with pelvic inflammatory disease [5] and on a single occasion has been found in the fallopian tube [6]. Serological studies have suggested a strong association between past infections with *M. genitalium* and tubal factor infertility [7]. It is therefore very likely that the organism could be considered as a sexually transmitted pathogen in women [8], and is responsible for at least some cases of urethritis, cervicitis, and pelvic inflammatory disease.

The optimal specimen type may vary depending on the sample preparation method. In women the use of more than one clinical specimen may also improve the diagnostic sensitivity such as supplementing a urine specimen with a cervical/vaginal swab [9].

This study was therefore carried out in both infertile and pregnant women in Lagos Metropolis, Nigeria to ascertain the presence of *M. genitalium* in them using High Vaginal Swabs and urine specimens.

In studies conducted in Denmark, the prevalence of infection was 2.3% and 1.1% in women and men respectively [10]. Tosh et al. [11] got a high prevalence of 13.6% in young sexually active women in the United States of America.

2. MATERIALS AND METHODS

2.1 Ethical Consideration

Prior to this study Ethical approval was obtained from Lagos University Teaching Hospital (LUTH) and 68 Nigerian Army Reference Hospital Yaba, Nigeria (68NARHY).

2.2 Study Centres and Population

The study centres where the samples (Urine and High Vaginal Swabs) were collected are the O&G Clinics of LUTH and 68 NARHY. Urine and High vaginal swabs were collected from 143 infertile women and 90 pregnant women. The specimens from married infertile women were part of a work-up for fertility investigations after failing to conceive for at least one year of unprotected sexual intercourse. The pregnant women were recruited from the Antenatal clinics of both hospitals. None of the subjects expressed any symptoms of genitourinary tract infection and were therefore considered asymptomatic.

2.3 Collection of Samples

High vaginal swab (HVS) specimens and 2 ml of first void urine from each subject were directly inoculated into 5 ml of *Mycoplasma* broth, mixed and transported to the laboratory at the Nigerian Institute of Medical Research, (NIMR), Yaba, Lagos.

2.4 Sample Preparation

Two milliliters of first void urine sample and HVS suspension from each of the subjects were put into a sterile 5 ml tube and centrifuged at 13,000 rpm for 5 minutes. The supernatant is discarded

and the cell pellets were resuspended in 50 µl of lysis buffer. After the homogenization, the cell pellets were incubated at 37℃ for 15 minutes so as to lyse the cells and degrade the proteins. The samples were them heated at 95℃ for 10 minutes to inactivate the protease and then centrifuged at 13,000 rpm for 5 minutes to sediment the cell debris. The supernatants were transferred into new microcentrifuge tubes. Positive and negative controls were set up along side the kit controls.

The standard strains of control organisms are; *Ureaplasma urealyticum* – ATCC 33175, *Mycoplasma genitalium* – ATCC 49123, *Mycoplasma hominis* – ATCC 23114, strain PG 21, and *Clostridium difficile* – ATCC BA – 2155, Strain LBM 0801058. The primers were obtained from NIMR, Yaba, Lagos.

2.5 PCR Reaction Setup

Twenty microliters of each of the universal PCR mix was transferred into a tube, 2.5 µl of the universal primers and 2.5 µl of the test samples were added and mixed gently using a voltex mixer. Positive and negative samples were set up alongside the controls. The tools were placed in a thermal cycler and were taken through the three steps of amplification procedure.

2.6 Gel Electrophoresis Protocol

Five microliters of the PCR product is added to 1.5 µl of loading buffer, and mixed thoroughly. The samples and a DNA marker (1000 bp ladder) were loaded on the 1.5% agarose gel. Positive and negative controls were set up alongside the kit controls. Electrophoresis was allowed to take place until the tracking dye has migrated 60-70% the dye length of the gel. The gel was then stained with ethidium bromide and viewed with ultraviolet illumination.

The kit detects 10^{-9} microgram quantities of target DNA. Samples that were positive for the presence of Mycoplasmas showed a distinct band at 434-468 bp. The positive control exhibited a 464 bp band while no visible band was noticed in the negative control lane.

2.7 PCR Molecular Species Identification

The PCR simultaneous detection and classification of *Mycoplasma genitalium* was carried out using the specific primer set My UU-forward 5^{l}-TGG AGT TAA GTC GTA ACA AG-3 and MyUU reverse r5^{l}-CTA AGA TGT TTC ACT TCA CC-3. The reaction was carried out in 25µl reaction mixture containing 1× PCR buffer, 1.5 mm Magnesium chloride. 0.2 nM of each dNTP, 1.25 u Taq DNA polymerase, 20 pMol of each primer and sterile distilled water was used to make up the reaction mixture. PCR was carried out in an Eppendiof Nexus thermal cycler with the following cycling parameters: an initial denaturation set up at 95℃ for 5 minutes followed by 30 consecutive cycles. After this, a final extension at 72℃ for 5 minutes was carried out.

The resulting PCR product was separated by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, visualized by UV transillumination and documented by photography. Image analysis was made by ImageQuantTL-V2003.03. The length of the amplification products were 495 bp for *Mycoplasma genitalium* positive samples (Table 1, Figs. 1-4)).

3. RESULTS

Table 1 shows the distribution of *M. genitalium* in both infertile and pregnant women in Lagos Metropolis. Out of the 143 urine samples from the infertile women, 2 representing 2.6% yielded *M. genitalium* while 2 (4.4%) of the 90 urine samples from pregnant women yielded *M. genitalium*. For the HVS samples (n=143), 5 (7.7%) were positive for *M. genitalium* in infertile women while 3(6.6%) out of the 90 samples from pregnant women were positive for *M. genitalium.*

Fig. 1 shows the simplified PCR products of positive urine and HVS specimens of infertile and pregnant women with standard control strains. The distinct clear bands represent standard control strains and randomly selected urine and HVS specimens.

The amplified PCR products of urine specimens from infertile women are shown in Fig. 2. Of the number tested, 2 samples (P2 and P27) were positive for *M. genitalium*. Fig. 3 shows amplified PCR products of urine specimens from pregnant women and samples P16 and P24 were positive for *M. genitalium* showing clear bands.

Fig. 4 shows the PCR amplified products of HVS specimens from infertile women. Samples 66v, 67v, 68V, 72V and 73V were positive for *M. genitalium* with clear bands. The amplified PCR

products of HVS specimens from pregnant women with *M. genitalium* are shown in Fig. 5. Samples P19V, P21V, and P29V were positive for *M. genitalium.*

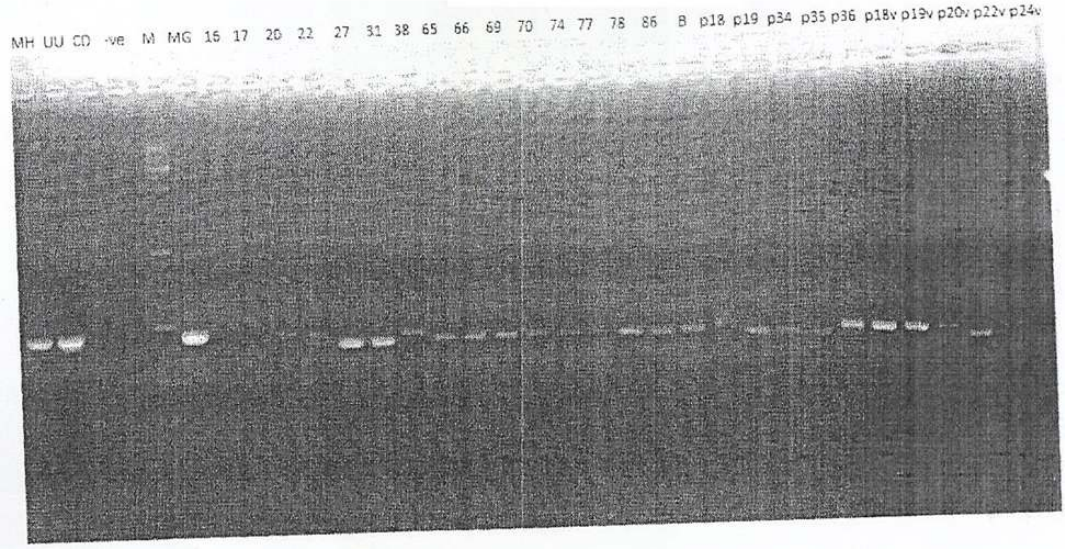

Fig. 1. Amplified PCR product of Mycoplasma positive urine and HVS specimens of infertile and pregnant woman with standard control strain

Keys: MH= Mycoplasma Hominis Standard Strain
UU= Ureaplasma urealyticum Standard Strain
CD = Clostridium difficile negative control
M= 100 BP DNA Molecular weight marker
MG= Mycoplasma genitalium Standard Strain

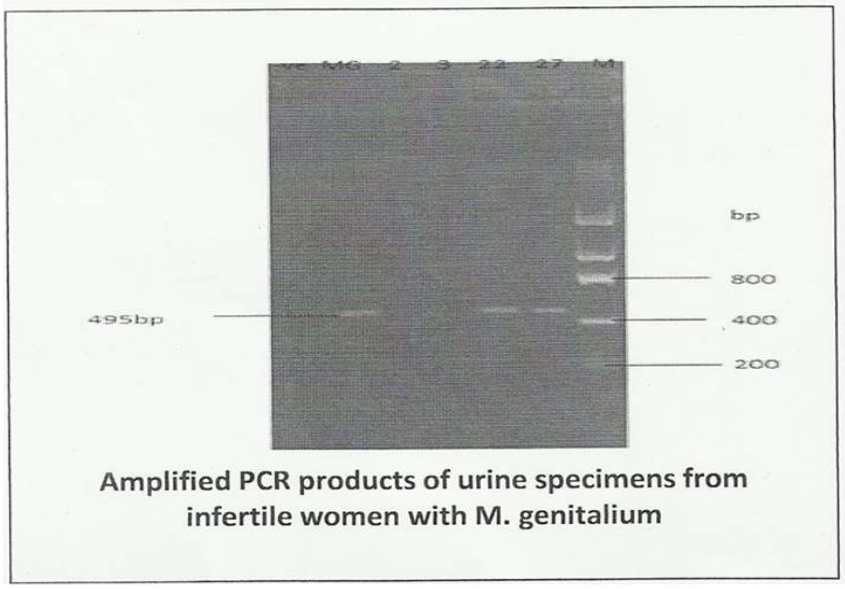

Fig. 2. Amplified PCR product of urine specimens from infertile women with *M. genitalium*

Keys: 2,3,22,27- Clinical urine samples infertile woman, -ve= Negative control
M.G= Mycoplasma genitalium, M= DNA molecular weight marker

Clinical samples	***Results***
22	*Positive*
27	*Positive*

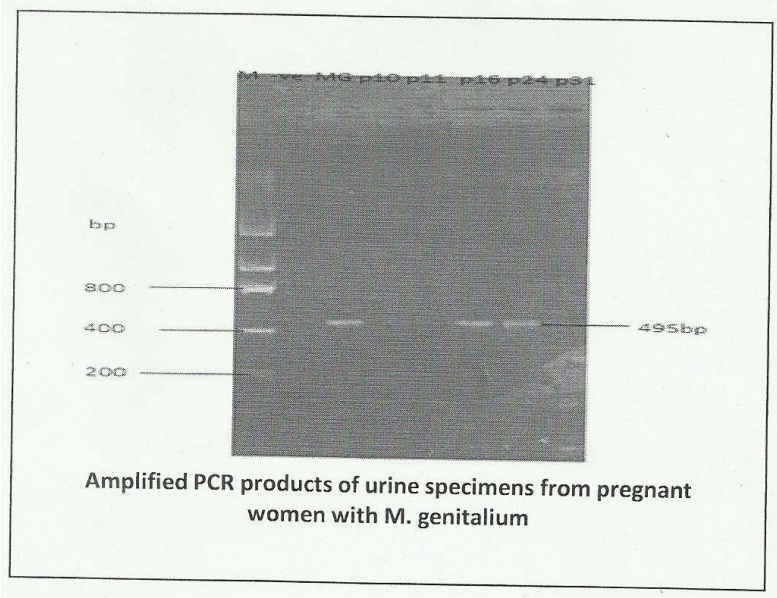

Fig. 3. Amplified PCR products of urine specimens from pregnant women with *M. genitalium*

Keys: P10, P11, P16, P24, P31= Clinical urine samples of pregnant woman, -ve= Negative control M.G= Mycoplasma genitalium, M= DNA molecular weight marker

Clinical samples	***Results***
16	*Positive*
24	*Positive*

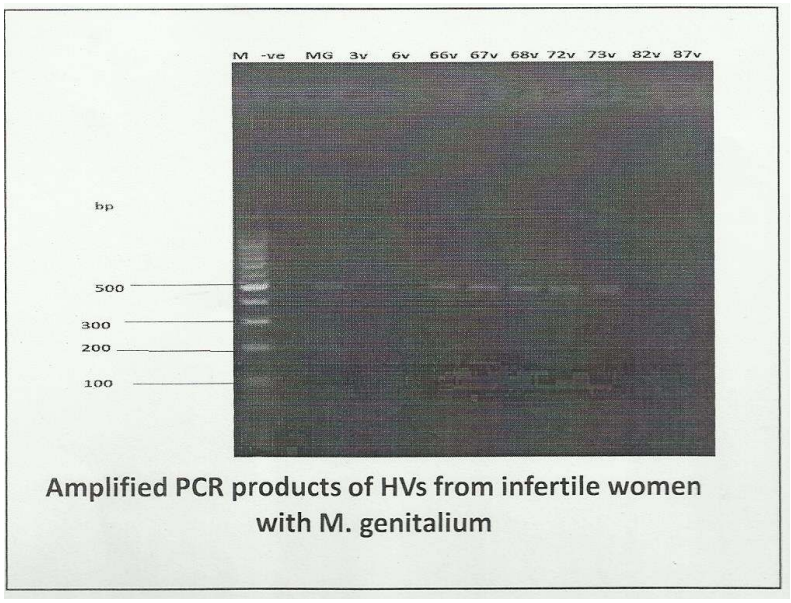

Fig. 4. Amplified PCR products of HVS from infertile women with *M. genitalium*

Keys: 3V, 6V, 66V, 72V, -73V, 82V, 87V,= Clinical HVS samples of infertile woman, -ve= Negative M.G= Mycoplasma genitalium, M= DNA molecular weight marker

Clinical samples	***Results***
66V	*Positive*
67V	*Positive*
68V	*Positive*
72V	*Positive*
73V	*Positive*

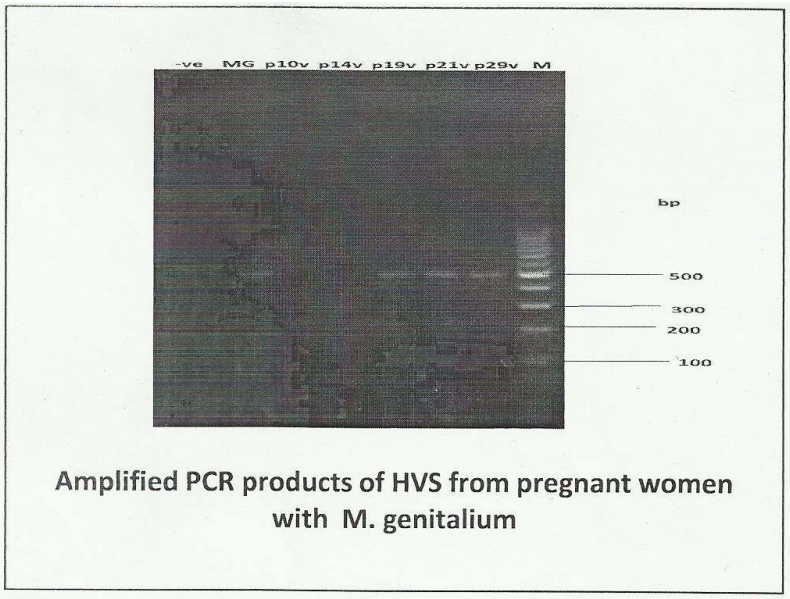

Fig. 5. Amplified PCR products of HVS from pregnant women with *M. genitalium*

Keys: P10V, P14V, P19V, P21V, P29V = Clinical HVS samples of pregnant woman, -ve= Negative control M.G= Mycoplasma genitalium, M= DNA molecular weight marker

Clinical samples	***Results***
P19V	*Positive*
P21V	*Positive*
P29V	*Positive*

Table 1. Distribution of *Mycoplasma genitalium* in Urine/HVS samples of infertile and pregnant women in Lagos, Nigeria

Infertile women (n= 143)	**No. sampled**	**No. positive**	**Percentage positive (%)**
Urine	143	2	2.6
HVS	143	5	7.7
Pregnant women (n= 90)	**No. sampled**	**No. Positive**	**Percentage Positive (%)**
Urine	90	2	4.4
HVS	90	3	6.6

4. DISCUSSION AND CONCLUSION

In the present study, a total of 7.7% of *M. genitalium* was recorded using PCR from the infertile women while 6.6% was recorded from the pregnant women. Diagnosis of *M. genitalium* infections is carried out exclusively using nucleic acid amplification tests (NAATs) due to the poor or slow growth of the organism on artificial culture media. *M. genitalium* has several virulence factors that are responsible for its pathogenicity. These include the ability to adhere to host epithelial cells with its adhesins, intracellular localization, the release of enzymes and the ability to evade the host immune response by antigenic variation [12].

In tandem with several other studies, *M. genitalium* has been linked to Non-gonococcal urethritis (NGU) [13,14,15]. It has been associated with cervicitis, pelvic inflammatory disease, adverse pregnancy outcome and infertility [13-15]. The detection of *M. genitalium* from both infertile and pregnant women shows that the organism inhabits the urogenital tract of women, and so can cause any of the afore-mentioned complications if the prevailing conditions are favorable.

The implication of the present study is that efforts should always be made when feasible to search for *M. genitalium* in infertility cases as it has been previously linked to infertility. There is little

information available on the role of *M. genitalium* in causing adverse pregnancy outcome, either as preterm labour, abortion or still birth. In one study [16], *M. genitalium* was detected in only 4% of mid-trimester vaginal swabs from 124 women delivering preterm. This is slightly higher than the prevalence in a normal population of pregnant women as estimated in another study [17] where only 0.7% of pregnant women in their first trimester were *M. genitalium* positive. In the present study, a prevalence of 5.5% was recorded, but did not monitor any pregnancy outcome, but this relatively high value is a cause for concern.

Thus, the prevalence of 7.7% and 6.6% of *M. genitalium* infection in 143 infertile and 90 pregnant women respectively is an indication that this organism should be routinely searched for in this category of women when no other etiologic agent has been implicated.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this paper and accompanying images.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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