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Molecular detection of *mycoplasma genitalium* among fertile and infertile women in Ibadan: a matched case-controlled study

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Abstract

Background: Mycoplasma genitalium, an emerging sexually transmitted pathogen of the Mycoplasmataceae family, has been reportedly associated with infertility. It is often asymptomatic and polymerase chain reaction (PCR) is the gold standard for diagnosis. This study is aimed to determine the prevalence of *M. genitalium* infection among fertile and infertile women in Ibadan using the conventional PCR technique.

Methodology: A cross-sectional hospital-based, matched case control study of 267 infertile and 135 consenting fertile women conducted between March and November 2015. Information on sociodemographic and behavioural characteristics were obtained by interviewer-administered questionnaire after which endocervical swabs were taken. The presence of *M. genitalium* infection was detected by Conventional PCR. All data were analyzed using SPSS version 20.0.

Results: The mean ages of the study and controlled groups were 33.8±5.7 and 31.9±4.5 years respectively ($p>0.05$). There was no difference in the socio-demographic and behavioural characteristics of the participants. The prevalence of *M. genitalium* was 16.1% among the infertile women and 2.2% among the controls ($p < 0.001$). This prevalence is similar among the types of infertility- primary or secondary.

Conclusion: The prevalence of *Mycoplasma genitalium* is significantly higher among infertile women thereby necessitating a call for appropriate microbiological screening. This will reduce the possibility of patients being under-investigated and misdiagnosed with subsequent inappropriate treatment. Routine screening for *M. genitalium* is therefore recommended in all women presenting with infertility.

Keywords: *Endocervical, infertility, Mycoplasma genitalium, PCR*

Résumé

Contexte: Mycoplasme genitalium, un émergeant agent pathogène sexuellement transmissible de la famille Mycoplasmataceae a apparemment été associé à la stérilité. Il est souvent asymptomatique et la réaction en chaîne de la polymérase (RCP) est la référence en matière de diagnostic. Cette étude vise à déterminer la prévalence de l'infection à *M. genitalium* chez les femmes fertiles et stériles d'Ibadan à l'aide de la technique conventionnelle RCP.

Méthodologie: Une étude transversale comparative cas-témoins basée en hôpital sur 267 femmes stériles et 135 femmes fertiles consentantes a été menée entre Mars et Novembre 2015. Les informations sur les caractéristiques sociodémographiques et comportementales ont été obtenues à l'aide d'un questionnaire administré par intervieweur, après quoi des prélèvements endo-cervicaux ont été effectués. La présence d'une infection à *M. genitalium a* été détectée par RCP conventionnelle. Toutes les données ont été analysées avec SPSS version 20.0.

Résultats: Les âges moyens des groupes d'étude et de contrôle étaient respectivement de 33,8 ± 5,7 ans et de 31,9 ± 4,5 ans ($p> 0,05$). Il n'y a pas de différence dans les caractéristiques sociodémographiques et comportementales des participants. La prévalence de *M. genitalium* était de 16,1% chez les femmes stériles et de 2,2% chez les témoins ($p <0,001$). Cette prévalence est similaire parmi les types d'infertilité - primaire ou secondaire.

Conclusion: La prévalence de *Mycoplasme genitalium* est significativement plus élevée chez les femmes infertiles, ce qui nécessite un appel à un dépistage microbiologique approprié. Cela réduira la possibilité que les patients subissent une sous-enquête et un diagnostic erroné avec un traitement subséquent inapproprié. Le dépistage systématique de *M. genitalium* est donc recommandé chez toutes les femmes présentant une stérilité.

Mots clés: *Endo-cervical, infertilité, Mycoplasme genitalium, RCP*

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Introduction

Mycoplasma genitalium, an emerging sexually transmitted pathogen of the Mycoplasmataceae family, has been described as a major cause of non-gonococcal urethritis and various inflammatory conditions in men and women respectively [1]. The pathogen has also been reported to be independently and significantly associated with pelvic inflammatory disease (PID) and tubal factor infertility [2-6]. The current global infertility rate has been documented as being between 15% and 20% with associated major economic burden on global healthcare industry [7, 8]. Tubal damage, resulting from tubal occlusion, is the most frequent cause of female infertility in most developing countries. The occlusions were usually caused by pelvic infection particularly from sexually transmitted infections (STIs) which accounted for 25% to 35% of cases [8]. According to Manhart *et al.*, asymptomatic carrier state of *M. genitalium* is a serious epidemiological problem because it enhances unnoticed transmission among sexual partners resulting in various adverse gynaecologic and reproductive events like infertility [9].

In sub-Saharan Africa, most studies on the role of infectious agents in infertility have been concentrated on *Chlamydia trachomatis* and *Neisseria gonorrhoea* among infertile women but these two agents alone had been unable to explain all the infectious causes of infertility [10-13]. It has also been noted that infections caused by *N. gonorrhoea* and *M. genitalium* were present in two-thirds of women with PID and have been associated with infertility [7]. In most instances, *M. genitalium* is asymptomatic, increasing the likelihood for "silent" PID and its infertility sequelae [14].

Some authors in separate studies equally reported that *M. genitalium* was more prevalent than *N. gonorrhoea* but less prevalent than *C. trachomatis* in the USA and that it was strongly associated with sexual activity [9, 15, 16]. Unfortunately, there is dearth of information on association of *M. genitalium* with infertility in Nigeria as specific laboratory investigations for its involvement in cases of unexplained infertility are rarely done [17]. Numerous risk factors have been associated with *M. genitalium* infection which included being uneducated, being unmarried or being a single parent, having migrant background, black ethnicity, smoking and having multiple sexual partners with the prevalence increasing by 10% for each additional sexual partner [18-20].

Isolation of *M. genitalium* had been hampered by difficulties associated with culture as well as its cross reactivity with *M. pneumonia* while the use of ELISA alone has been fraught with several reports of false positive results. However in 2010, two authors, Kenny and Razin, in separate documentations stated that polymerase chain reaction (PCR) is a very sensitive and specific method for its diagnosis [21, 22]. The main objective of our study is to determine the prevalence of *M. genitalium* among women diagnosed with infertility in Ibadan using Nucleic acid Amplification technique (NAAT) and comparing the results with those of women with proven fertility in the same environment. The data from this study may help in the need to establish strategic preventive programme for "at risk" population and this may contribute to reduction in the prevalence of infertility in Nigeria.

Materials and methods

This was a cross-sectional hospital-based study, conducted between March 1 and November 30 2015, in which a total of 399 consenting women were recruited at the University College Hospital (UCH) and Adeoyo Maternity specialist hospital (AMSH), Ibadan. The UCH is an 880 bedded tertiary health centre located in Ibadan city and serves as referral centre for other hospitals in the country while AMSH is a secondary care centre mostly serving the lower and middle class level patients within the Ibadan metropolis.

From the first two consenting patients that presented on the selected clinic day, one patient was randomly selected using balloting technique. Afterwards every alternate patient was selected until the desired sample, a maximum of 10 for each clinic day, was met. Using interviewer-administered questionnaire, information was obtained on participants' sociodemographic and behavioural characteristics. Endocervical swabs were also taken to test for *M. genitalium*. Non-consenting women and those who recently used antibiotics were excluded from the study.

Written informed consent was obtained after careful and detailed explanation of the concept of the study to each patient before inclusion. Ethical standards were followed in the handling, storage and disposal of specimens. The Ethics committee of the University of Ibadan, University College Hospital Ibadan and Oyo state government approved the study before commencement.

Collection of endocervical swab

Endocervical swabs were collected under aseptic condition for each recruited woman using sterile Copan® flocked eNat cervical swabs with 2mls of nucleic acid preservative (Copan Italia Diagnostics) and stored at "20° C until processing [23].

Biosafety issues and universal precaution

Mycoplasma genitalium has been described as a Bio safety level–2 organism and was therefore treated as such. Personal protective equipment including laboratory coats, masks, goggles and sterile latex gloves were used. Work surfaces were decontaminated daily, before and after work, with 10% hypochlorite solution.

DNA extraction from endocervical specimen

The Jena Bioscience Bacteria DNA preparation kit (Jena Bioscience GmbH, Germany) was used according to the manufacturer's instruction [24]. About 1ml of endocervical specimen in eNat preservation medium was centrifuged at 10000 ×g for 1 min. The supernatant was discarded while 300 µL of lysis buffer and 2 µL of RNase A were added to the pellet. The mixture was vortexed vigorously for 30 to 60 sec, 8 µL of proteinase K was added to it followed by incubation at 60°C for 10 min. After allowing to cool for 5 min, 300µL of binding buffer was added to the mixture and this was vortexed briefly. The solution was placed on ice for 5 min and then centrifuged for another 5 min at 10000×g. A spin column was placed into a 2 mL collection tube and the mixture was discarded into it. Lysate was pipetted directly into the spin column, centrifuged for 1 min at 10000 ×g and the flow-through was discarded. About 500µL of washing buffer was added into the spin column, centrifuged for 30 sec at 10000×g and the flow-through was discarded (this was done twice). The spin column was centrifuged again at 10000×g for 1 min to remove the residual washing buffer. The 2 mL tube was discarded; the spin column was placed in the elution tube and 40 to 50 µL of elution buffer was added into the center of the spin column. It was incubated at room temperature for 1 min and centrifuged at 10000 ×g for 2 min. The extracted DNA was stored at "20°C.

PCR assay

Primer sequences used for *M. genitalium Mgpa (major adhesion protein) gene* primer were Mgp-F 5'aag tgg agc gat cat cat tac taa c-3' Mgp-R 5' ccg tgg tta tca tac ctt ctg a- 3' [25].

PCR was set up with reaction mixture comprised of 5µL of DNA extract, 0.40µL of primers (forward and reverse), 10.60µL of PCR water, 4µL of Master Mix (reaction buffer B, MgCl2, dNTPs, blue and yellow dye). Each tube containing the reaction mixture was sealed and briefly centrifuged before amplification in PCR machine. Amplification was done using the following protocol: initial denaturation at 95°C for 5 min, then 30 cycles of denaturation at 95°C for 40 sec, annealing at 56°C for 30sec, extension at 72°C for 40secs and final extension at 72°C for 5min with each amplification run containing distilled water as the negative control. After amplification, electrophoretic separation of PCR products was performed on 1.5% agarose gel stained with ethidium bromide, and visualized by Ultraviolet illumination.(Uvitec, UK).

Electrophoresis

About 10iL of DNA ladder (Solis BioDyne, Estonia) at 100bp gradient was placed at one end of wells of 1.5% agarose gel stained with 2il ethidium bromide. Thereafter, 10iL of the amplified product was added into each well on the agarose gel, placed into the electrophoretic tank and switched on at 100 voltage for 60 minutes. The tray containing the agarose gel and bands were transferred to a bio-imaging system and results were read. Bands corresponding to 495 bp on the DNA ladder were documented as positive for *M. genitalium*.

Data analysis

Data analysis was done using the SPSS version 20 software. We determined the means and standard deviation for quantitative variables and proportions for qualitative variables. Chi square test was done for appropriate categorical variables with level of statistical significance set at $P<0.05$.

Results

The study aimed to determine the prevalence of *M. genitalium* among fertile and infertile women in Ibadan using PCR for molecular detection. It is a hospital-based study in which 399 consenting women were recruited. The sample size calculated was 390 (260 cases and 130 matched controls in the ratio of 2:1) but the number with inconsistent information is 6 and the total is 399. The mean ages of the study participants were similar in the 2 groups (33.9±5.7 versus 31.9±4.5 years respectively, $p>0.05$): Most of the respondents were married and had tertiary level of education. Across the groups, there was no difference in their marital status, education and religion. These sociodemographic parameters were presented in Table 1. Majority of the respondents, 156 (58.6%) in the study population had secondary infertility while 118 (44.4%) had previous voluntary termination of pregnancies.

Mycoplasma genitalium was detected in 43 (16.2%) infertile women and 3 (2.3%) of the control (P<0.001, table 2; Figure 1). The odds of an infertile woman having *M. genitalium* infection was more than eight times more likely than a fertile counterpart (Odds Ratio, OR = 8.36, 95% confidence interval 2.54 – 27.47; p = 0.0005). Of the 156 women with secondary infertility, 27 had positive results for *M. genitalium* giving a prevalence rate of 17.3% while 16 women in the primary infertility group had positive results – a prevalence of 14.5%. However, these findings were not statistically significant (P>0.05; Table 3).

Among the respondents, *M. genitalium* infection was significantly associated with the participants who were single or divorced and were of low socioeconomic status. However, there were no associations between *M. genitalium* and past history of sexually transmitted infections and condom use (Table 4).

Discussion

Our study aimed to dete
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prevalence rates for *M. geni*

Table 1: Socio-demographic factors of the respondents

Socio-demographic Factors	Cases Frequency (%)	Control Frequency (%)
Age Group (Years)		
20 – 24	9 (3.4)	5 (3.8)
25 - 29	51 (19.2)	39 (28.6)
30 – 34	90 (33.8)	50 (36.8)
35 – 39	71 (26.7)	35 (26.3)
≥ 40	45 (16.9)	6 (4.5)
Marital Status		
Married	253 (95.1)	130 (97.7)
Single	12 (4.5)	3 (2.3)
Divorced	1 (0.4)	0 (0.0)
Education		
Primary	19 (7.1)	3 (2.3)
Secondary	92 (34.6)	18 (13.5)
Tertiary	154 (57.9)	112 (84.2)
Others	1 (0.4)	0 (0.0)
Religion		
Christianity	166 (62.4)	110 (82.7)
Islam	100 (37.6)	24 (17.3)

Table 2: Relationships between the prevalence of *mycoplasma genitalium* among the study group

Variable	Groups Study group	Control group	Chi–S
PCR results: Positive	43	3	16.82
Negative	223	130	

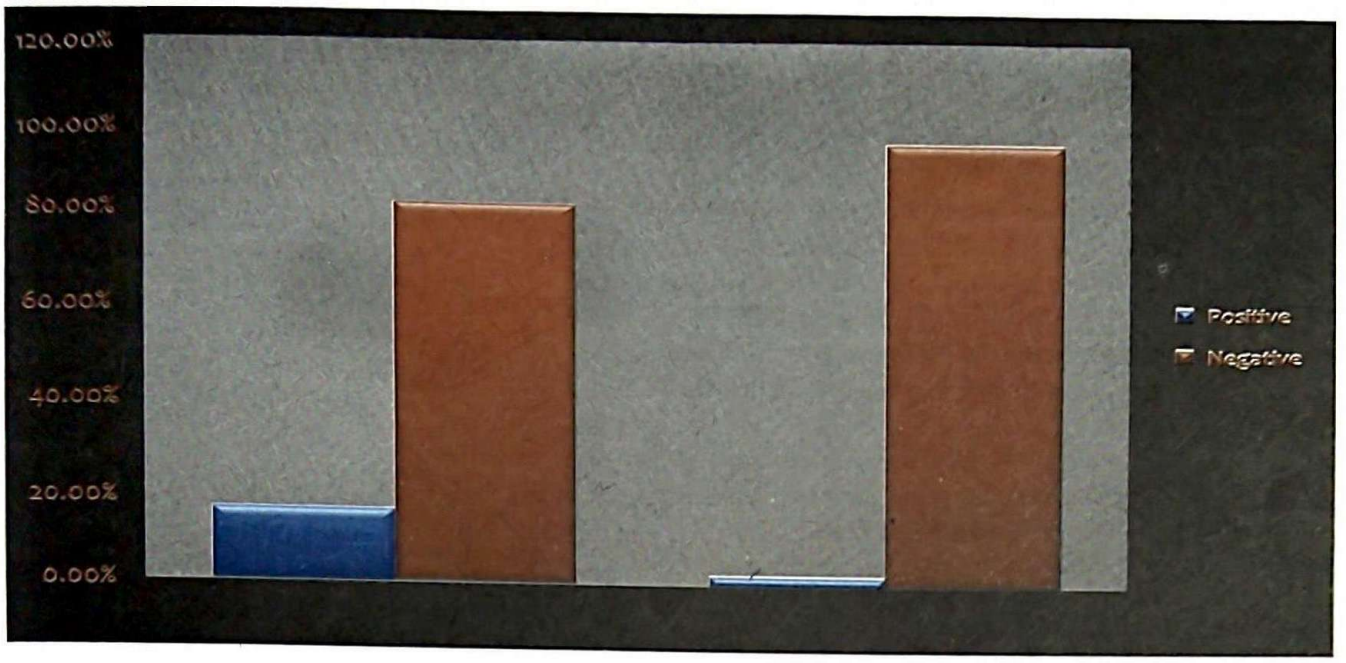

Fig. 1: Prevalence of *mycoplasma genitalium* among the study and control groups

Table 3: Prevalence of *mycoplasma genitalium* among women with primary and secondary infertility.

Variable		Type of infertility		RR (95% Confidence Interval)	P – value
		Primary	Secondary		
PCR results:	Positive	16	27	0.88 (0.58 – 1.34)	> 0.05
	Negative	94	129		

Table 4: Factors associated with *mycoplasma genitalium*

Variable	M. genitalium		Chi – Square	p-value
	Yes (%)	No (%)		
Grouped Age (in years)				
20 – 29	14 (23.0)	46 (77.0)	3.52	0.172
30 – 39	21 (13.0)	140 (87.0)		
≥ 40	8 (17.8)	37 (82.2)		
Family type				
Single/Divorced	10 (76.9)	3 (23.1)	38.72	**<0.001**
Monogamous	31 (14.2)	188 (85.8)		
Polygamous	2 (5.9)	32 (94.1)		
Monthly Income (in naira)				
< 18,000	15 (29.4)	36 (70.6)	8.17	**0.004**
≥ 18,000	28 (13.0)	187 (87.0)		
Past history of Gonorrhoea / STI				
Yes	19 (12.8)	81 (81.0)		
No	19 (12.8)	130 (87.2)	4.08	0.130
Don't know	5 (29.4)	12 (70.6)		
Use of condom				
Yes	9 (24.3)	28 (75.7)	2.11	0.146
No	34 (14.8)	195 (85.2)		

ranging from 22% to 31.9% in studies done using serological assay [8, 28, 29]. We however noted that serological assays are limited in sensitivity because of cross reaction between the antibodies of *M. genitalium* and *M. pneumonia*. In view of this limitation, many authors favour PCR, particularly the real time PCR, which has excellent sensitivity and specificity [30-34]. In India, Rajkumar *et al.*, reported an overall prevalence rate of 16% among patients with infertility using real time PCR – a similar value to our findings although on the contrary, they obtained higher values for subjects with primary infertility [35].

The higher prevalence of *M. genitalium* among infertile women illustrates the possibility of a link between the pathogen and female infertility. There are evidences for *M. genitalium* cervicitis and PID thus indicating that this organism has potential to cause ascending infections which can lead to infertility [2, 36-38]. Although some studies have established the sexual transmission mode of *M. genitalium*, there were no documented relationships with previous history of STIs (sexually transmitted infections) which was also corroborated in this study [15, 18, 20, 39]. However, the lack of association between previous history of STI and *Mycoplasma genitalium* in this study may be due to recall bias, deliberate non-disclosure or presence of mild symptom which was ignored.

As corroborated by our findings and previous studies, *M. genitalium* has been shown consistently to have a strong association with female infertility and should be considered as an actiologic factor. The use of PCR to improve the sensitivity and specificity of the diagnosis further strengthen this assertion. We are of the opinion that these findings will lead to a reduction in the possibility of patients being under-investigated and/or misdiagnosed followed by subsequent inappropriate/ inadequate treatment. Routine identification of *M. genitalium* is therefore indicated in all women presenting with infertility.

Limitation of the study

Our study was limited by non-use of real time PCR which is known to be more sensitive than Conventional PCR used in this study thereby implying that the prevalence may be higher than we reported. We were also unable to determine the presence of *M. genitalium* among the spouses of our respondents which would have enabled us to assess the possibility of the mode of transmission.

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