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Seroprevalence of *Mycoplasma genitalium* among Fertile and Infertile Women at Murtala Muhammad Specialist Hospital, Kano

Bakori, H, S[1]., Kumurya, A. S[2]. and Idris, A. M[3*].
[1]Department of Microbiology, Federal University, Dustinma, Katsina State, Nigeria
[2]Department of Medical Laboratory Science, Bayero University, Kano, Nigeria
[3]Department of Medical Microbiology and Parasitology, Bayero University, Kano, Nigeria
correspondence:: amikibiya@gmail.com; +234-7038228730.

Abstract
Mycoplasma genitalium is an emerging sexually transmitted pathogen with potential implications for reproductive health. This study aimed to investigate and compare the seroprevalence of M. genitalium among fertile and infertile women to provide insights into its potential association with fertility outcomes. A cross-sectional study was conducted involving a total of 90 women, including 31 fertile and 59 infertile women. Serum samples were collected and tested for the presence of M. genitalium-IgG antibodies using ELISA detection techniques. Data on demographic characteristics, including age and education, were also collected. Prevalence rates of M. genitalium-IgG antibodies were calculated for both fertile and infertile groups, and a comparative analysis was performed. The seroprevalence of Mycoplasma genitalium-IgG antibodies was higher among fertile women (90.3%) compared to infertile women (84.7%). The largest age group was 25-29 years, representing 33.3% of participants, with 18 (20.0%) infertile and 12 (13.3%) fertile women. The majority of participants had secondary education. Risk factors, including sharing innerwear, type of toilet, awareness of sexually transmitted diseases, number of sexual partners, and family setting, showed no statistical significant associations with the presence of M. genitalium IgG ($p > 0.05$). The study revealed a notably higher seroprevalence of M. genitalium-IgG antibodies among fertile women compared to infertile women and recorded the non-significant association between M. genitalium infection and possible risk factors observed.
Keywords: *Mycoplasma genitalium, seroprevalence, fertility, infertility, IgG antibodies*

INTRODUCTION

Mycoplasma genitalium (*M. genitalium*) is a sexually transmitted bacterium that can cause various reproductive health issues in both men and women (Zhang *et al.*, 2023). In infertile women, *M. genitalium* has been identified as a potential pathogen that can contribute to reproductive problems (Smolarczyk *et al.*, 2021). Studies have shown a potential association between *M. genitalium* infection and infertility in women. *M. genitalium* can lead to inflammation and damage in the reproductive organs, including the fallopian tubes, cervix, and uterus (Yu *et al.*, 2023). This inflammation can interfere with normal fertility processes, such as ovulation, fertilization, implantation, and maintenance of pregnancy (Ojo *et al.*, 2023). The exact mechanisms by which *M. genitalium* infection affects fertility are still being investigated, but it is believed that the Antibiotics like Azithromycin and Moxifloxacin are commonly used to treat *M. genitalium*, but bacterium can cause damage to the reproductive tissues, disrupt the normal functioning of the endometrium, and impair sperm transport through the fallopian tubes (Deng *et al.*, 2022). Additionally, *M. genitalium* infection has been associated with conditions like pelvic inflammatory disease (PID), which can further lead to infertility (Ravel *et al.*, 2021).

Diagnosing *M. genitalium* infection in infertile women can be challenging since it is a relatively newly recognized pathogen, and routine testing for *M. genitalium* is not yet widely available (Gnanadurai and Fifer, 2020). However, there are molecular tests, such as polymerase chain reaction (PCR), that can detect *M. genitalium* DNA in genital samples and provide a definitive diagnosis. Treating *M. genitalium* infection is essential for both the resolution of symptoms and the prevention of long-term complications (Workowski and Bachmann, 2022).

antibiotic resistance is a growing concern (Jensen *et al.*, 2022). Therefore, it is important

to follow treatment guidelines and consider retesting to ensure successful eradication of the infection.

MATERIAL AND METHODS

Study area

The research was conducted at Murtala Muhammad Specialist Hospital (MMSH), situated in the Kano Municipal Local Government Area (LGA) of Kano State, located in the Northwest geopolitical zone of Nigeria. Kano State has a population exceeding 13 million and comprises 44 Local Government Areas covering around 20,760 square kilometers (NBS, 2018). MMSH is recognized for its affordability and accessibility to individuals with moderate socioeconomic backgrounds in both Kano City and nearby LGAs.

Study population

The study population comprised consented fertile and infertile women attending Murtala Muhammad Specialist Hospital and aged ≥ 18 years.

Inclusion criteria

Only women participants who have consented and agreed to participate in the study, with a history of infertility, and pregnant women attending infertility and antenatal clinics were included in the study.

Study design

This is a prospective cross-sectional hospital-based study.

Ethical consideration

Ethical approval was obtained for this study from the Kano State Ministry of Health (Approval Code: MOH/OFF/797/T.I/703), ensuring that the research adhered to ethical standards and guidelines.

Data Collection

The data for this study were collected using simple structured questionnaires to obtain the participants' biodata, sociodemographic data, and medical history.

Sample size determination

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where: n = number of size for finite population
n = number of samples for infinite population
Z = statistic for level of confidence at 95% = 1.96
P = Previous prevalence (6.0%).
d = allowable error of 5%, (0.05)

$$n = \frac{1.96^2(0.06)(1-0.06)}{0.05^2}$$

n = 86.6

A total of 90 participants, including 59 infertile and 31 fertile, were selected for the study

Result Determination

Positive control average OD ≥ 1.00,
Negative control average OD ≤ 0.100
CUT OFF value = negative control average + 0.15
If OD < CUT OFF: Negative for Human Mycoplasma genitalium-IgG
If OD ≥ CUT OFF: Positive for Human *Mycoplasma genitalium*-IgG

Sample collection

Following data collection, sterile 2ml sterile syringes were used to obtain blood samples using the venipuncture method. The samples were collected into appropriate collection tubes labeled with the participant code and handled with aseptic techniques to prevent contamination.

The collected blood samples were then transported in a cold box containing ice packs to the Microbiology laboratory of MMSH for processing and analysis.

Sample preparation and processing

The collected whole blood was left undisturbed at room temperature (approximately 10-20 minutes) to allow it to clot naturally. After clot formation, the clot was removed by centrifugation at a speed of 2000-3000 revolutions per minute (rpm) for 5 minutes. After centrifugation, the resulting serum was carefully collected and transferred into appropriate labeled storage tubes. The serum samples were then stored at a temperature of -20ºC in a freezer.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum was tested for *M. genitalium* antibodies by ELISA detection techniques (Sunlog Biotech, 2018).

Procedure

In a Micro ELISA strip plate, Three wells were used as negative controls, two as positive controls, and one as a blank. 50 µl of negative and positive controls were added to the respective control wells. For the sample wells, 40 µl of sample dilution buffer and 10 µl of the sample were mixed by gently shaking. The plate was sealed and incubated at 37°C for 30 minutes. A washing buffer was prepared by diluting 20 ml of concentrated buffer with 580 ml of distilled water. After incubation, the plate was washed by aspirating and refilling the wells with wash solution, allowing it to stand for 30 seconds, and repeating this process 5 times. Next, 50 µl of HRP-Conjugate reagent was added to each well except the blank, and the plate was incubated again at 37°C for 30 minutes. After repeating the wash process, 50 µl of chromogen solutions A and B were added to each well, gently mixed, and incubated for 15 minutes at 37°C. The reaction was stopped by adding 50 µl of stop solution. The absorbance was read at 450 nm using a microtiter plate reader.

Data Analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc, Chicago, IL, USA). Chi-square was used to examine the associations between demographics and prevalence of the infection. Percentage and table were used to show the distribution and prevalence of the infection. The level of statistical significance was set as $p< 0.05$

RESULTS

A total of 90 participants, including 59 infertile and 31 fertile women, were enrolled in this study, and an overall prevalence of 86.7% *M. genitalium* IgG was obtained. A higher percentage 90.3% of *M. genitalium* IgG positive was found in fertile women than 84.7% in their counterpart infertile women (Table 1).

The highest proportion of the participants, 30 women (33.3%) were among the age range 25 – 29 years, that comprised of 18 (20.0%) infertile and 12 (13.3%) fertile women. Participants aged 40 – 44 years had the least number of participants, with 2 (2.2%) infertile women. The secondary education group has the highest proportion of both fertile and infertile individuals (Table 2).

Table 3 shows the distribution of the *M. genitalium* possible risk factors. Risk factors, including sharing innerwear, type of toilet, awareness of sexually transmitted diseases, number of sexual partners, and family setting, tend to show statistical significant difference with the presence of *M. genitalium* IgG with probability value of >0.05.

Table 1: Seroprevalence of *M. genitalium* IgG antibodies among women Attending Murtala Muhammad Specialist Hospital, Kano

		Distribution of *M. genitalium* IgG Antibodies	
Test Status of *M. genitalium*	**Number Screened No (%)**	**Infertile Women No (%)**	**Fertile Women No (%)**
Positive	78 (86.7)	50 (84.7)	28 (90.3)
Negative	12 (13.3)	9 (15.3)	3 (9.7)
Total	**90 (100.0)**	**59 (100.0)**	**31 (100.0)**

Table 2: Seroprevalence of *M. genitalium* IgG antibodies in Relation to Demographics among women Attending Murtala Muhammad Specialist Hospital, Kano

Demographic factors	**No. Tested No (%)**	**Infertile women No (%)**	**Fertile women No (%)**
Age (Years)			
15 – 19	7 (7.8)	3 (3.3)	4 (4.4)
20 – 24	13 (14.4)	6 (6.7)	7 (7.8)
25 – 29	30 (33.3)	18 (20.0)	12 (13.3)
30 – 34	26 (28.9)	18 (20.0)	8 (8.9)
35 – 39	12 (13.3)	12 (13.3)	0 (0.0)
40 – 44	2 (2.2)	2 (2.2)	0 (0.0)
Total	**90 (100.0)**	**59 (65.6)**	**31 (34.4)**
Education			
Primary	13 (14.4)	11 (12.2)	2 (2.2)
Secondary	47 (52.2)	24 (26.7)	23 (25.6)
Tertiary	18 (20.0)	12 (13.3)	6 (6.7)
Other	12 (13.3)	12 (13.3)	0 (0.0)
Total	**90 (100.0)**	**59 (65.6)**	**31 (34.4)**

Table 3: Seroprevalence of *M. genitalium* IgG antibodies in Relation to Risk Factors among Women Attending Murtala Muhammad Specialist Hospital, Kano

Risk Factors for *M. genitalium*	No. Tested No(%)	Infertile No(%)	Fertile No(%)	*P-value*
Sharing Inner Wear				
Yes	1 (1.1)	1 (1.1)	0 (0.0)	1.000
No	89 (98.9)	58 (64.4)	31 (34.4)	
Type of Toilet				
Pit	22 (24.4)	18 (20.0)	4 (4.4)	0.106
Water Cistern	68 (75.6)	41 (45.6)	27 (30.0)	
Aware of STD				
Yes	21 (23.3)	14 (15.6)	7 (7.8)	1.000
No	69 (76.7)	45 (50.0)	24 (26.7)	
Number of Sexual Partners				
One	90 (100.0)	59 (65.6)	31 (34.4)	-
More than One	0 (0.0)	0 (0.0)	0 (0.0)	
Family Setting				
Monogamy	53 (58.9)	31 (34.4)	22 (24.4)	0.142
Polygamy	37 (41.1)	28 (31.1)	9 (10.0)	

DISCUSSION

This study found an overall prevalence rate of 86.7% for *Mycoplasma genitalium* IgG antibodies, which is significantly higher than the results of other studies. Stafford *et al.* (2021) reported a prevalence rate of 5.7% among pregnant women in the southwestern United States, while Moridi *et al.* (2020) recorded an 11.3% prevalence in a systematic review and meta-analysis conducted in Iran. Additionally, Nye *et al.* (2020) found a rate of 4.0% in women with bacterial vaginosis in the United States, and Rekha *et al.* (2019) reported a prevalence of 3.4% from the peritoneal fluid of fertile and infertile women at a medical college in Jaipur, India, based on Canadian data. The discrepancies in results between these studies could be a result of the study population's varying standard of living and geographic location. While other studies with lower incidence were conducted in industrialized nations with good standards, study which had a high prevalence, was conducted in poor resource and emerging nations.

A higher *M. genitalium*-IgG antibody prevalence (90.3%) was observed among fertile women compared to infertile women (84.7%). This result is in contrast with what was documented elsewhere; for example, Moridi *et al.* (2020) reported a prevalence rate of 12.7% among infertile women and 3.0% among fertile women. Rekha *et al.* (2019) reported 6.2% for infertile women compared to 0.6% for fertile women. Idahl *et al.* (2015) reported 5.4% and 1.6% among infertile and fertile women, respectively. These differences can be attributed to a variety of factors, including study populations, sample size, geographic regions, methodology, and diagnostic criteria.

The study's finding that primary infertility is most prevalent among women aged 25-29 years contrasts with the established notion of peak fertility in the early and mid-twenties (Ashraf *et al.*, 2013). This discrepancy might be attributed to the study's population, where early marriage is common, in contrast to developed countries where women often delay childbearing.

Furthermore, the study found no significant association between risk factors like sharing personal effects, personal hygiene, and toilet type and the presence of *M. genitalium* infection among infertile women. This contradicts Chukwuka *et al.* (2013) findings, where these factors were significantly linked to *M. genitalium* colonization in adolescents. The disparity could be attributed to differences in the age groups studied and, potentially, the prevalence of *M. genitalium* within these populations.

CONCLUSION

Our study reveals a notably higher seroprevalence of *M. genitalium*-IgG antibodies among fertile women compared to infertile women, in contrast to previous findings. These findings suggest that there is a need for comprehensive research involving larger sample sizes, diverse populations, and standardized diagnostic methods is crucial to better understand the relationship between *M. genitalium* infection and fertility. Such insights could contribute to improved reproductive health management and interventions.

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