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Molecular Detection of *Neisseria gonorrheae* and *Chlamydia trachomatis* among Infertile Male Clients attending some Health Facilities in the Northwest, Nigeria

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Abstract

The purpose of this study was to molecularly detect and evaluate the prevalence of Chlamydia trachomatis and Neisseria gonorrhoeae infections among infertile men that attended some health facilities in some states of the Northwest, Nigeria using a multiplex molecular method as well as note the possible impact of C. trachomatis and N. gonorrhoeae infections on semen parameters. A total of 383 infertile male semen samples were collected for this study. C. trachomatis and N. gonorrhoeae infections were molecularly detected in the semen samples using the multiplex PCR method. Semen parameters, risk factors, and age were also analyzed and collated. The results indicated that a total of 28 and 21 semen samples were found positive for C. trachomatis and N. gonorrheae, respectively, with a relatively lower prevalence of 7.3% and 5.5%, respectively. Those with primary infertility have the highest infections, with those with secondary infertility exhibited the least infection. In conclusion, our study demonstrated a moderately medium prevalence of C. trachomatis and N. gonorrheae infection based on the multiplex PCR detection method. The study submits that C. trachomatis and N. gonorrheae infection could possibly impair male fertility through increased sperm DNA damage.

Keywords: Chlamydia trachomatis, Infertile Males, Multiplex PCR, Neisseria gonorrhoeae, Semen samples

INTRODUCTION

Infertility is defined as the inability of couples to reproduce after a year of unprotected sexual intercourse (Albert *et al.*, 2014; Hassan *et al.*, 2019; WHO, 2023). Male infertility is a public world health problem affecting about 10–15% of couples, which accounts for half of the infertile cases (Albert *et al.*, 2014, WHO, 2023). The cause of male infertility has been multifactorial in which the role of urinary tract infections has been identified to be associated in recent reproductive health services. Chlamydia trachomatis, Neisseria gonorrhoeae, mycoplasma species (Mycoplasma genitalium and Mycoplasma hominis), ureaplasma species (Ureaplasma urealyticum and Ureaplasma parvum) and Treponema pallidum are the major urinary tract infections.

Precise mechanisms of urinary pathogens inflicting male fertility capability remain complex and unknown. The process of triggered inflammation by urinary pathogens leading to the deterioration of spermatogenesis and obstruction of the seminal tract could be partly related to the cause of infertility. Also, the process of apoptosis is related to inflammatory conditions, which could cause the impairment of semen parameters, though no established literature backs the association between infections and semen parameters (Gimenes *et al.*, 2014; Kim *et al.*, 2017).

There are two types of infertility: Primary and secondary infertility. Primary infertility is defined as a couple's inability to conceive after at least one year of sexual intercourse without the use of contraception. Couples who have conceived at least once but were unable to conceive again have secondary infertility (Abu Khanjar and AL-Azari, 2022). Some research work says that urinary tract infections

implicated by some infectious pathogens include; bacteria, viruses, fungi, and protozoa, are responsible for 15% of male infertility (Hassan *et al.*, 2019; Abu Khanjar and AL-Azari, 2022). Microorganisms may influence the male reproductive system through motile sperm agglutination, decreasing acrosome reaction capacity, and changing cell morphology, or indirectly by releasing reactive oxygen species produced by infection-induced inflammation (Oghael *et al.*, 2020; Vadim, 2024), which has been linked to sperm DNA fragmentation and male infertility (Oghbael *et al.*, 2020). Any man can have asymptomatic urinary tract infections, such as chlamydia and Neisseria infections, which can be detected in urine and seminal fluid (Hassan *et al.*, 2019). Infections of the urinary tract are a common cause of infertility in men, and several bacteria have been implicated in infertility in men in several ways. Infections cause serious havoc on reproduction in several ways. Retardation to the spermatogenesis process, sperm malfunction, and urinary tract obstruction are some of these processes. Chlamydia trachomatis and N. gonorrheae pathogens are the most common bacteria linked to male genital infections (Abu Khanjar and Al-Azawi, 2022). The World Health Organization reports about 370 million UTI infections per year. The pathogenic bacteria N. gonorrhea and C. trachomatis are responsible for 87 million of them. The illness is one of the world's most deadly and rapidly spreading diseases. Moreover, the second most common sexually transmitted disease is gonorrhea and Chlamydia. Predisposing factors include younger age, a new sex partner, a sex partner with concurrent partners, many sex partners, a history of gonorrhea infection, and having other sexually transmitted illnesses are the most common risk factor.

The fundamental contemporary diagnostic method of urinary tract pathogens has been the bacterial cultural method, which is prone to so many errors (personnel, reagents, and equipment), time-consuming, and with less sensitivity. The molecular method of detecting these pathogens directly from the samples based on nucleic acid amplification has been widely used in many research-related works, has proven reliable, and has relatively high sensitivity and specificity (Cook *et al.*, 2016).

A semen sample was used in the detection of C. trachomatis and N. gonorrhoeae (Mohammed and Mutalib, 2022). Multiplex PCR method was adopted for the amplification testing method based on isothermal amplification of pathogens RNA, which has provided an accurate and rapid detection of the urinary pathogens (Cook *et al.*, 2016; Mohammed and Mutalib, 2022). To our knowledge, there is no data available published regarding the prevalence of C. trachomatis and N. gonorrhoeae, in infertile men using direct semen samples for the detection of pathogens. Thus, this study aimed to molecularly detect and observe the prevalence of C. trachomatis and N. gonorrhoeae,

MATERIALS AND METHODS

Study Area

The study covered selected states of the Northwest geopolitical region of Nigeria. The region consist of seven states in the Northwestern Nigeria; Kano, Jigawa, Kaduna, Katsina, Kebbi, Sokoto, and Zamfara. The weather is usually dry, and the temperature drops at night. The occupational tendency of inhabitants of the region is largely agricultural.

The Northwest region has a population of about 35,786,944 million and a total land mass of 216,029 km^2 (Ado, 2016; Kehinde, 2019; NPC/FMH, 2020).

The region shares boundaries with Bauchi State in the North East from Kano State, in the North with Niger Republic with Jigawa State, and Niger State through Zamfara State from Sokoto; it also borders with Niger Republic in the Northern part of the State. From Kebbi State, the region borders with Niger and the Benin Republic; through Katsina, Kaduna, and Jigawa State, the region borders the Niger Republic in the north, FCT Abuja, Nasarawa, Plateau, Bauchi, and Yobe states respectively (Ado, 2016; NPC/FMH, 2020). The region is multicultural and multi-ethnic but majorly dominated by Hausa Fulani and many other minority ethnic groups (Ado, 2016; Kehinde, 2019; NPC/FMH, 2020).

Study Population

The study population consisted of suspected male infertile patients attending health facilities within the selected States in the Northwestern region of Nigeria, who consented and gave their semen samples for the research. The selected States and health facilities are Kano State: Aminu Kano Teaching Hospital (AKTH Kano) and Murtala Specialist Hospital Kano, Kaduna State: Barau Dikko Teaching Hospital Kaduna and Dr. Gwamna Awan General Hospital Kakuri, Kaduna and Jiigawa State: Federal Medical Center Dutse and Rasheed Shekoni Specialist Hospital(now Teaching Hospital).

Inclusion criteria

Adult married male patients with a suspected case of either primary or secondary infertility who can produce semen and are attending any

of the health facilities within the selected states.

Exclusion criteria

Adolescents, singles, those who cannot produce sperms (oligospermia), and those who declined consent.

Sample Size Determination

A total of three hundred and eighty (380) samples were used for the research. This was obtained using the standard formula provided by Riley *et al*. (2020). Using the prevalence rate of infertility of 40 -50 % in Nigeria (Udia and Enokpe, 2017; Westmann, 2018) and a precision of 5 % (with 15% confidence).

Formula: $n = \frac{z^2 p(1-p)}{d^2}$

$n = Z^2 P (1- P)$

d^2

Where:

n = Sampling size

p = Prevalence rate

Z = Standard normal distribution of 95% confidence limit = 1.96

d = Absolute desired precision at 5% = 0.05

Hence, $n = \frac{1.96^2 \times 0.45(1-0.45)}{0.05^2}$ = 380

However, 383 samples were collected for equality and precision.

These samples were not equally distributed because of the variation in the number of clients attending the selected health centres varies. Therefore, based on the variation in the inflow of clients in these facilities, the following sample allocations were obtained: AKTH Kano – 91, BDTH Kaduna – 67, Federal Medical Center Birnin Kudu – 67, Murtala Mohammed Specialist Hospital Kano – 67, Rasheed Shekoni Specialist Hospital Dutse, Jigawa State – 46 and Gwamna Awan General Hospital Kakuri Kaduna State – 46.

Ethical Considerations

Ethical approvals were obtained from the ethical committees of the various health institutions as attached in appendices iii – x with the following reference numbers: MOH/Off/797/T.1/1331, MOH/ADM/1744/VOL.1/754, MOH/SEC.3/S/808/1, FMC/HREC/APP/CLN/001/1/184, AKTH/MAC/SUB/12A/P-3/VI/2763, BDTH/HREC/RF: 19 - 00015 and RSSH/GEN/226/V.1/11. Verbal and written consents were obtained from the volunteers using the consent form attached in Appendix i. All clients received an explanation (both in Hausa and English) of the study before obtaining informed consent. Well-structured questionnaires attached in appendix ii (explained in Hausa for those who could not understand, read, or write) were administered to all clients before samples were collected (appendix 8).

Sample Collection and Storage

All samples were collected by masturbation into sterile graduated wide-mouth containers following 3 days of sexual abstinence; equally plain condoms were given to those who couldn't restrain self-control during masturbation/unable to use masturbation in order not to compromise the semen volume. After liquidation, a conventional analysis was performed according to the World Health Organization guidelines (WHO, 2010).

The parameters measured are the volume of the ejaculate (ml), the sperm concentration (x106/ml), the motility (%), and the morphology (% of normal forms).

Bacteriological Analysis

Macroscopic and Microscopic examination

The semen samples were observed for liquefaction for 1 hour after ejaculation to prevent dehydration, which may affect the semen volume and quality. The viscosity of the sample was estimated by gently aspirating it into a wide-bore (1.5 mm diameter) plastic disposable pipette, allowing the semen to drop by gravity and observing the length of the thread. The color of semen was checked, normal liquefied semen sample incorporated a homogeneous, grey-opalescent look.

The samples were directly collected into a graduated glass or plastic container with a wide mouth, and the volumes were directly observed from the graduations (with 0.1 mL accuracy). Similarly, the pH was measured using a pH meter within 1 hour of the collection, whereby the pH paper was dipped into the semen sample for 30 seconds, and after 30 seconds, the color of the impregnated zone was compared with the pH standard calibration chart.

The sample was thoroughly mixed to obtain a heterogeneous liquefied sample before taking representative semen for analysis, sperm motility, vitality, concentration, and morphology. A standard volume (10 µl) of semen was placed onto a clean glass slide and then covered with a coverslip of 22 × 22 mm with a chamber depth of approximately 20 µm, taken care to avoid the formation of air bubbles, then allowed to stand for 5minutes for the sperm cells to settle and then was examined microscopically under light microscope, aside from spermatozoa, other cells were looked for as some of which may be clinically relevant, such

as leukocytes and immature germ cells, the latter 2 together brought up as "round cells."

Extraction of Genomic and Microorganisms' DNA

Two hundred microliters (200µL) of semen sample was transferred to a microcentrifuge tube, and 200µL of biofluid and cell buffer (red) 20µL of proteinase K was added. The mixture was vortex for 10 – 15 seconds and then incubated at 55^{0}C for 10 minutes. One (1) volume of genomic binding buffer was added to the digested sample and was vortex for 10 -15 seconds (equal volume; 420µL Genomic binding buffer to the 420µL digested sample). The mixture was transferred to a Zymo-spin™ IIC-XLR Column in a collection tube and Centrifuged at >12000 × for 1 minute. The collection tube was discarded with the flow through. Then 400µL DNA pre-wash buffer was added to the spin column in a new collection tube and was centrifuged at > 12000 × g for 1 minute, and the collection tube was emptied. Then 700µL g-DNA wash buffer was added to the spin column, Centrifuged at 12000 × g for 1 minute, and the collection tube was emptied. Then 200µL g-DNA wash buffer was added to the spin column and was centrifuged at 12000 × g for 1 minute, and the collection tubes with the flow through were discarded.

The spin column was transferred to a clean microcentrifuge tube, and 100µL DNA Elution buffer was added directly to the matrix and was incubated for 5 minutes at room temperature, then was centrifuged at maximum speed for 1 minute to elute the DNA. The eluted DNA was used partly immediately and stored at -20^{0}C.

Molecular detection of *Chlamydia trachomatis*

The *Chlamydia trachomatis* gene was detected by PCR using a pair of primers targeting the conserved region of the *C. trachomatis,* as described in Table 3.1. Each tube was made to a final volume of 20µL comprising of 4µL of DNA template, 10µL of 2xEasyTaq® PCR Supermix (TransGen, Beijing, China), 4µL of molecular grade water, and 2µL of each of the forward and reverse primers of the *omp1* (Table 3.1). The negative control tube comprised of the same constituents except for the DNA which was replaced with molecular-grade water.

The mixtures were loaded into the PTC100 Peltier thermocycler (MJ Research, USA), and the PCR condition was set as follows: pre-denaturation at 95^{o}C for 5 minutes; 40 cycles of denaturation at 95^{o}C for 30 seconds, annealing at 60^{o}C for 30 seconds and extension at 72^{o}C for 30 seconds; then a final extension at 72^{o}C for 5 minutes. After the PCR, 5µL of the amplicons were separated and detected by gel electrophoresis containing 2µL of Ethidium bromide on 1% TBE buffer with 100V for 1 hour. The bands were visualized using a UV Tran illuminator, captured, and analyzed by the Image computerJ software (Schroeder *et al.*, 2021).

Molecular Detection of *Neisseria gonorrhoeae*

The *Neisseria gonorrhoeae* gene was detected by PCR using a pair of primers targeting the conserved region of the *C. trachomatis,* as described in Table 1. Each tube was made to a final volume of 20µL comprising of 4µL of DNA template, 10µL of 2xEasyTaq® PCR Supermix (TransGen, Beijing, China), 4µL of molecular grade water, and 2µL of each of the forward and reverse primers of the *N. gonorrhoeae* (Table 1). The negative control tube comprised of the same constituents except for the DNA which was replaced with molecular-grade water.

The mixtures were loaded into the PTC100 Peltier thermocycler (MJ Research, USA), and the PCR condition was set as follows: pre-denaturation at 95^{o}C for 5 minutes; 40 cycles of denaturation at 95^{o}C for 30 seconds, annealing at 55^{o}C for 30 seconds and extension at 72^{o}C for 30 seconds; then a final extension at 72^{o}C for 5 minutes. After the PCR, 5µL of the amplicons were separated and detected by gel electrophoresis containing 2µL of Ethidium bromide on 1% TBE buffer with 100V for 1 hour. The bands were visualized using a UV Tran illuminator, captured, and analyzed by the ImageJ computer software (Schroeder *et al.*, 2021).

Table 1: Primers and probes for PCR

Primer	Primer sequence (5' - 3')	Band size (bp)
C. trachomatis (*ompA*)	F: ATG AAA AAA CTC CTT GAA ATC G R: GAA CTA AGG ATG CCT CTA TTG	800
N. gonorrhoeae (*ProA*)	F: CCG GAA CTG GTT TCA TCT GAT T R: CAA GCC GAT AAA GGA TTC CCT	295

(Simoni *et al.*, 1999, Chen *et al.*, 2022; Zhai *et al.*, 2022)

Statistical Analysis

Data analysis was performed using SPSS version 22.0 for Windows Software Package (SPSS Inc., Chicago, IL, USA). Data were expressed as percentages and mean ± SD. To identify the independent association between the various

seminal parameters and selected variables, independent t test.

RESULTS

The distribution of sociodemographic factors among the study population indicated that those between the ages of 29 – 37 had the highest number of study subjects, 207(54.0%), while ages 58-68 had the least number of subjects, 3(0.8%). The tribe had the Hausa/Fulani with the highest population of subjects, 254(66.3%), and Igbos with the lowest number of subjects, 25(6.5%). The occupation had farmers with the highest number of 160(41.8%), while others had the lowest number of subjects, 34(8.9%). Type of marriage had monogamy with the highest number of subjects, 294(76.8%), while polygamy had the least number of subjects, 89(23.2%). Years in marriage had those who have spent more than six years into their marriages with the highest number of subjects, 116(30.3%), while those who are 1 year old into their marriage had the least of subjects, 18(4.7%) Table 2.

Table 2: Socio-demographic characteristics of the participants

VARIABLES	FREQUENCY	PERCENTAGES (%)
AGE		
18 – 28	60	15.7
29 – 37	207	54.0
38 – 48	89	23.2
49 – 57	24	6.3
58 – 68	3	0.8
TRIBE		
Hausa/Fulani	254	66.3
Yoruba	36	9.4
Igbo	25	6.5
Others	68	17.8
OCCUPATION		
Civil servants	110	28.7
Farmers	160	41.8
Artisans	79	20.6
Others	34	8.9
TYPE OF MARRIAGE		
Monogamy	294	76.8
Polygamy	89	23.2
YEARS IN MARRIAGE		
1	18	4.7
2	38	9.9
3	54	14.1
4	68	17.8
5	89	23.2
6+	116	30.3

The relationship between risk factors and semen parameters, independent t-test, and one-way ANOVA were used to test for significance between the two groups. The mean SD was also determined as seen from the Table 3 below. There was no significant relationship recorded among those who smoke, consume alcohol, infection, and drug use ($p > 0.05$). However, a significant result was recorded between fertility, pH, and viscosity, as well as diagnosed infections, viscosity, and liquefaction respectively with mean SD of 1.14±0.35 and 1.25±0.43; P = 0.02 (P < 0.05).2.05±1.75 and 1.64±1.38; P= 0.03, 1.10±0.30 and 1.19±0.39; P= 0.06, 1.00±0.00, 1.25±0.53, 1.40±0.63, 1.00±0.00, 1.83±0.41, 1.50±0.71, 1.06±0,00, 1.00±0.00, 1.00±0.00; P=0.006, 1.25±0.50, 1.90±1.63, 1.85±1.65, 1.00±0.00, 4.67±1.20, 1.00±0.00, 1.29±0.49, 1.50±0.71and 3.50±2.89; P=0.004($p > 0.05$) respectively.

Table 3: Distribution of the risk factors in relation to semen parameters among the study population

Risk factor	n	Semen parameters: Sperm count	Vol.	Motility	Morph.	PH	Liq.	Vis.
Smoking								
Yes	38	4.68±2.26	2.66±0.71	1.76±0.94	1.24±0.43	1.13±0.34	1.39±0.68	1.71±1.45
No	345	4.23±2.22	2.76±0.59	1.76±0.93	1.30±0.46	1.18±0.38	1.28±0.53	1.96±0.69
P-value		**0.24**	**0.32**	**0.98**	**0.41**	**0.49**	**0.20**	**0.33**
Alcohol Consumption								
Yes	79	4.63±2.09	2.81±0.48	1.75±0.94	1.27±0.45	1.10±0.30	1.30±0.63	1.80±1.48
No	304	4.19±2.25	2.73±0.62	1.76±0.92	1.30±0.46	1.19±0.39	1.28±0.53	1.97±1.71
P-value		**0.11**	**0.31**	**0.89**	**0.52**	**0.06**	**0.79**	**0.38**
Drug use								
Normal	369	4.28±2.24	2.75±0.59	1.75±0.92	1.30±0.46	1.18±0.38	1.28±0.54	1.91±1.64
Hard	12	4.08±1.88	2.67±0.78	1.83±1.03	1.33±0.49	1.08±0.29	1.42±0.67	2.25±1.26
Snuff	2	5.50±0.71	3.00±0.00	3.00±0.00	1.00±0.00	1.00±0.00	1.50±0.71	3.50±1.54
P-value		**0.71**	**0.74**	**0.16**	**0.63**	**0.57**	**0.61**	**0.32**
Fertility								
Primary	276	4.28±2.19	2.74±0.60	1.74±0.92	1.31±0.46	1.14±0.35	1.29±0.55	2.05±1.75
Secondary	107	4.28±2.31	2.77±0.58	1.82±0.95	1.25±0.44	1.24±0.43	1.29±0.55	1.64±1.38
P-value		**1.00**	**0.73**	**0.42**	**0.26**	**0.02**	**0.96**	**0.03**

Table 4: Distribution of the risk factors in relation to semen parameters among the study population

Risk factor	n	Semen parameters: Sperm count	Vol.	Motility	Morph.	PH	Liq.	Vis.
Past Infection								
Yes	226	4.42±2.17	2.75±0.60	1.79±0.93	1.30±0.46	1.16±0.37	1.31±0.55	1.95±1.60
No	157	4.07±2.28	2.75±0.59	1.71±0.92	1.29±0.46	1.19±0.39	1.26±0.55	1.91±1.67
P-value		**0.13**	**0.95**	**0.41**	**0.94**	**0.43**	**0.44**	**0.8**
Diagnosis								
Yes	4	4.00±2.45	2.50±0.58	1.75±0.96	1.00±0.00	1.00±0.00	1.00±0.00	1.25±0.50
No	303	4.23±2.23	2.75±0.58	1.75±0.92	1.28±0.45	1.18±0.38	1.25±0.53	1.90±1.60
Staph.	53	4.49±2.16	2.72±0.66	1.66±0.94	1.42±0.50	1.13±0.34	1.40±0.63	1.85±1.62
Staph./E.C.	2	4.50±3.54	3.00±0.00	1.00±0.00	1.50±0.71	1.00±0.00	1.00±0.00	1.00±0.00
Chlamydia	6	2.67±2.25	2.33±1.03	2.67±0.82	1.33±0.52	1.50±0.55	1.83±0.41	4.67±1.20
Gonorrhea	2	6.00±1.41	3.00±0.00	2.00±1.41	1.50±0.71	1.50±0.71	2.50±0.7	1.00±0.00
Gono/*Staph.*	7	5.29±1.50	3.00±0.00	2.00±1.00	1.00±00	1.14±0.39	1.29±0.49	1.57±1.13
Syph/Staph	2	7.00±0.00	3.00±0.00	1.50±0.71	1.50±0.71	1.00±0.00	1.50±0.71	1.50±0.71
Chla/Staph.	4	3.75±2.06	3.00±0.00	2.50±1.00	1.50±0.58	1.00±0.00	1.25±0.50	3.50±2.89
p-values		**0.28**	**0.55**	**0.20**	**0.21**	**0.33**	**0.006**	**0.004**

Twenty-eight (28) samples were found with *Chlamydia trachomatis* given a percentage of 7.3, while Twenty-one (21) samples were detected with *Neisseria gonorrheae*, with the least percentage of 5.5 Tables 5. Primary infertility has the highest number of both *Chlamydia trachomatis* 20 (71.4) and *Neisseria gonorrheae* 13 (61.9) compared with those with secondary infertility with 8(28.6) for *C. trachomatis* and 8(38.10 for *N. gonorrheae*.

Table 5: Frequency of *Chlamydia trachomatis and Neisseria gonorrhoeae* among the study population

Parameter	**no**	**Frequency**	**Percentage (%)**
Chlamydia trachomatis	383	28	7.3
Neisseria gonorrhoeae	383	21	5.5

Table 6: Frequency of *Chlamydia trachomatis* and *Neisseria gonorrheae* primary and secondary infertile population

INFERTILITY	No. examine	**Bacterial pathogens**				
		C tracomatis	*N neisseria*	**□2**	**df**	**p-value**
1 fertility	277	20(71.4)	13(61.9)	**0.012**	**1**	**0.91**
2 fertility	106	8(28.6)	8(38.1)	**1.205**	**1**	**0.27**
p > 0.05 – not statistically significant						

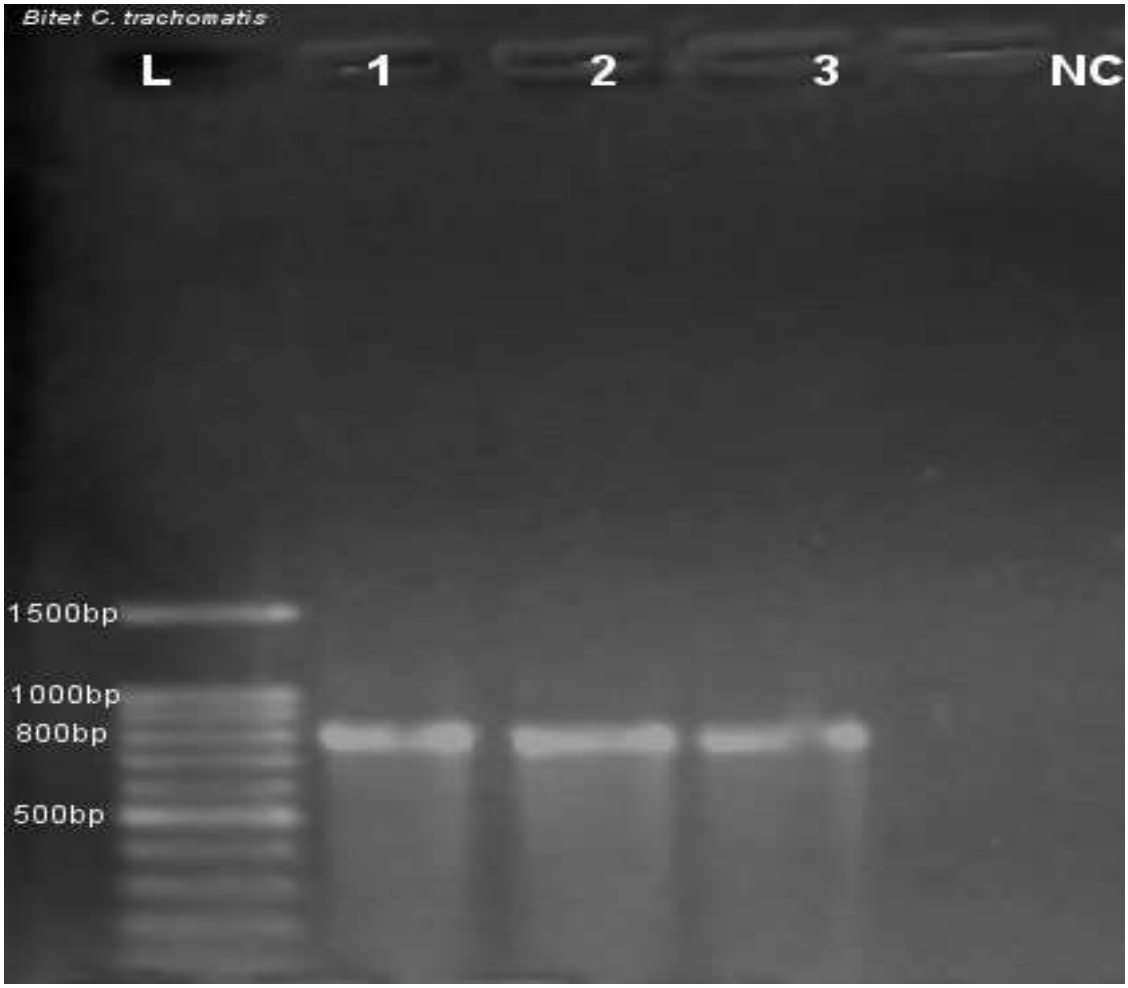

Plate 1: A representative of Agarose gel electrophoresis patterns showing PCR amplification products from *Chlamydia trachomatis* isolates. L: A molecular weight size marker (100bp+). Lane 1-3: positive for *omp1* gene as indicated by 800 base pairs. NC: negative control

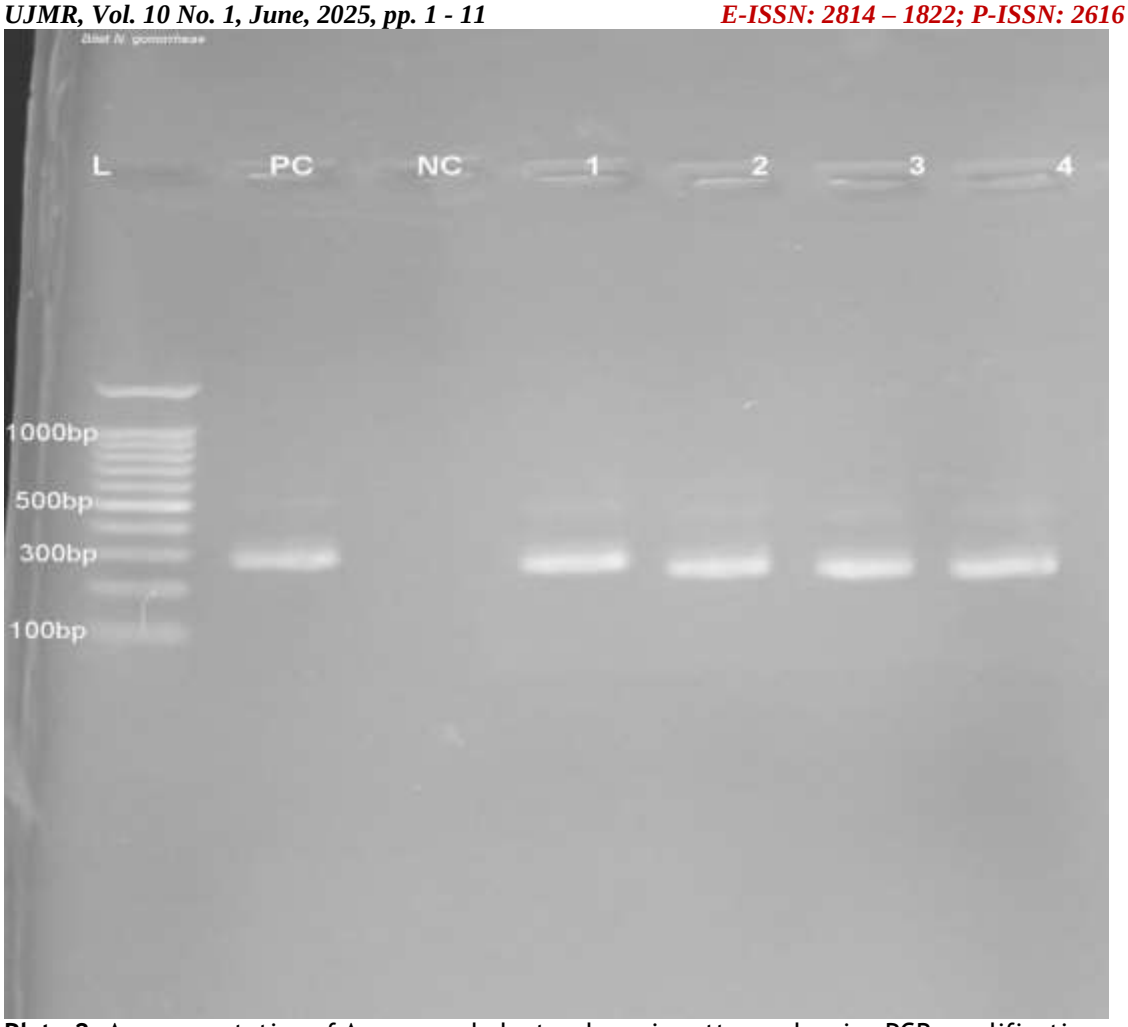

Plate 2: A representative of Agarose gel electrophoresis patterns showing PCR amplification products from *Neisseria gonorrhoeae* isolates. L: A molecular weight size marker (100bp+). PC: positive control. NC: negative control. Lane 1-4: positive for *proA* gene as indicated by 295 base pairs.

DISCUSSION

Infertility is a common reproductive public health problem in our country and the world at large. Therefore, the need to find out the male partner's impact on the infertility challenge in our society cannot be over-emphasized. The clinical subjective semen analysis still plays a critical role in the diagnosis and management of male infertility. However, précised diagnosis of infertility rarely depends on semen analysis results. There is a significance interrelationship between the semen parameters of fertile and infertile men. Hence, there is a need to assess the sperm nuclear integrity in our society.

Male urinary tract infection (UTI) has caused a lot of discussion in relation to male infertility, and it is believed to contribute to approximately 15% of male infertility (Abursarah *et al.*, 2013; Qing *et al.*, 2017; Bitet *et al.*, 2021, Ahmadi *et al.*, 2023). *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the most prevalent and asymptomatic urinary tract bacterial pathogens globally studied in most literatures. The clinical asymptomatic nature of these pathogens makes identifying the infection easily and equally difficult, the chances of contamination with other organisms and culturing more complex (Qing *et al.*, 2017; Duvuru *et al.*, 2022; Mohammed *et al.*, 2022).

Our study detected *N. gonorrhoeae* and *C. trachomatis* infections in 7.3% and 5.5%, respectively, out of the 383 infertile male subjects. This outcome is similar to the study outcome of 6.5% for *N. gonorrhoeae* and *C. trachomatis* varied between 0.4 -42.3% by Qing *et al.* and Abursarah *et al.* (Abursarah *et al.*, 2013; Qing *et al.*, 2017). Other findings within Nigeria reveal some variation in prevalence from place to place, such as *N. gonorrhoeae* 1.4% in Lagos, 1.3% in Ilorin, 5.2% in Calabar, 3.2% in Abuja, 5.0% in Port Harcourt and 16.3% in Kaduna, 2.0% in Oweri (Nsofor and Eletuoh, 2017, Mbah *et al.*, 2022). In another study in 2014 by Nwankwo and Sadiq (2014), Lagos showed a high prevalence rate of 51% and 18.2% with a wide margin, Jos 51.6%, South Eastern

Nigeria 40.7%, Zaria and Kano in Northwestern Nigeria 38.3% and 18.4% respectively. Furthermore, 44.4% was reported in Zaria, 80.0% in Ibadan, and 13.3% in Benin City (Nwankwo and Sadiq, 2014; Keshinro *et al.*, 2016). Some African countries have a prevalence of 16.3% in Hawasa Ethiopia Majnooni *et al.*, (2022), 2.7% in Tunisia by Hammami *et al.*, (2014). Global prevalence is 2.2%, with the highest prevalence in Africa at 5.0% (Chemaitelly *et al.*, 2021). Generally speaking, there is a variation in the spread of *N. gonorrhoeae* among infertile subjects.

On the other hand, *C. trachomatis* also has a variable prevalence of 9.6% in Kano, Nigeria (Nwankwo *et al.*, 2014), Delta 46.5%, South East 51%, Jos 51.6%, South West 41%, Northern Nigeria 38.3% and Benin City 13.3% (Bamikole *et al.*, 2020). Other part of the world, Africa inclusive, had 10.0% in Ethiopia (Ahmadi *et al.*, 2023), 15.3% in Iran and in Babol, Iran, 11.6%, and Tunisia 43.3% (Qing *et al.*, 2017, Bamikole *et al.*, 2020), Brazil .12.3%, USA 5%, and Europe 1.7 17% (Bamikole *et al.*, 2020). Variation in prevalence could result from sample size and detection methods (Mawak *et al.*, 2011; Nwankwo and Sadiq, 2014).

The differences in prevalence reports may be due to differences in laboratory methods, geographical and cultural characteristics of the states and countries, and equally important is variation in sample size (Ahmadi *et al.*, 2023). The implications of urinary tract infections on male infertility are still under study, including the effect on semen parameters fertility capacity in the area of aided reproduction. Several studies outcomes fail to address the possible correlation between *N. gonorrhoeae* and *C. trachomatis* infections and semen paraters (Sallami *et al.*, 2014; Qing *et al.*, 2017; Duvuru *et al.*, 2022, Mohammed and Abdul Mutalab, 2022), including this present study. While many others reported a decrease in semen volume, sperm count, morphology, and motility with *C. trachomatis* and *N. gonorrhoeae* infections but semen quality deteriorations probably caused by *C .trachomatis* and *N. gonorrhoeae* were not fully cleared with some reported a detrimental effect of bacteriological pathogens on male fertility power as others reported changes in semen parameters (Qing *et al.*, 2017, Duvuru *et al.*, 2022). Hence, the difference in the criteria for male infertility diagnosis and bacterial pathogens detection techniques in different studies may contribute to the reports' wide variation.

CONCLUSION

Using multiplex PCR to detect *C. trachomatis* and *N. gonorrhoeae* provides a sensitive, faster and accurate technique to detect the presence or absence of these urinary tract and symptomless pathogens in the semen. The result of this current study reveals that *C. trachomatic* and *N. gonorrhoeae* infections probably spread in infertile males and could cause a decrease in sperm DNA integrity, motility, morphology and count.

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