MOLECULAR EVALUATION OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE* INFECTIONS AND THEIR CO-INFECTION AMONG WOMEN WITH INFERTILITY ISSUES AT GYNEACOLOGICAL CLINICS IN ADO – EKITI, NIGERIA

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Accepted for publication 21/5/2025

ABSTRACT

Background: *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are obligate intracellular bacteria that cause urethritis, cervicitis, salpingitis, pelvic inflammatory disease, ectopic pregnancy, painful urination, abnormal and unusual increase discharge from the vagina and penis. Due to the similarities in these microorganisms' clinical presentations, one can be taken for the other.

Aim: This study evaluated the level of chlamydia and gonorrhoeae infections and their co-infection among women attending fertility clinics in Ado–Ekiti, Ekiti State.

Methods: 115 urine and 115 high vagina swab samples (230 samples) were collected from women attending selected fertility clinics. Rapid kits specific for *C. trachomatis* and *N. gonorrhoeae* were used and quantitative polymerase chain reaction (qPCR) was carried out to further test for these microorganisms in both urine and high vagina swabs samples.

Results: A total of 36 (31.3%) and 39 (33.9%) were positive to *C. trachomatis* and *N. gonorrhoeae* respectively when tested with rapid test kit using urine sample; 37 (32.2%) and 49 (42.6%) were positive to *C. trachomatis* and *N. gonorrhoeae* respectively when urine samples were tested with qPCR; 13 (11.3%) were positive *C. trachomatis* when HVS samples were tested with qPCR, 51 (44.3%) were positive to *N. gonorrhoeae* with HVS samples when tested with qPCR. A total of 19 (16.5%) showed co–infection of *C. trachomatis* and *N. gonorrhoeae* with rapid kit using urine; 23 (20%) showed co–infection of these microorganisms when tested with qPCR and 9 (7.8%) showed co–infection from HVS samples tested with qPCR. Co–infection of *C. trachomatis* and *N. gonorrhoeae* were statistically significant ($P< 0.05$).

Conclusion: This study has indicated the need for inclusion of the screening of these organisms routinely especially in fertility centers and gynecological clinics. This will further help in combating the havoc that can be caused by these silent microorganisms.

Keywords: Infertility, *C. trachomatis*, *N. gonorrhoeae*, co–infection, women.

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INTRODUCTION

Chlamydia trachomatis is the most common sexually transmitted bacterial infections worldwide, and women carry the major burden of the disease, as well as the potential source of infection to their partners. The incidence of Chlamydial infections in women has increased dramatically from 79 to 467 per 100,000 between 1987 and 2003[1]. According to the World Health Organization report (WHO) [2], 101 million Chlamydial infections are detected annually worldwide. The clinical presentation, course, complications and late sequelae of *C. trachomatis* closely resemble *Neisseria gonorrhoeae* infection. There are four recognized species of Chlamydia: *C. trachomatis*, *C. psittaci*, *C.*

pneumoniae and *C. pecorum*. *Chlamydia trachomatis* includes the agents of trachoma, lymphogranuloma venereum (LGV), urogenital tract disease, and inclusion conjunctivitis[3]. No attempt has been made to comprehensively review the biology, pathogenesis, or epidemiology of *C. trachomatis* infections, although several excellent reviews have been published on these subjects[4]. The prevalence of *C. trachomatis* infection in sexually active adolescent women, the population considered most at risk, generally exceeds 10%, and in populations of women, the prevalence can reach 40%. The prevalence of *C. trachomatis* infection ranges from 4 to 10% in asymptomatic men and from 15 to 20% in men attending STD clinics[5]. Chlamydial infections in newborns occur as a result of perinatal exposure with approximately 65% of babies born from infected mothers becoming infected during vaginal delivery[6]. The biggest challenge to the control of Chlamydial disease is that as many as 70 to 80% of women and up to 50% of men who are infected do not experience any symptoms[7]. This results in a large reservoir of unrecognized, infected individuals who are capable of transmitting the infection to sexual partners. Contributing to this challenge is the fact that immunity following infection is thought to be type specific and only partially protective. Therefore, recurrent infections are common. Evidence suggests that the risk of developing sequelae such as ectopic pregnancy or infertility increases with each successive episode of infection[7, 2]. Although most infections caused by *C. trachomatis* in women are asymptomatic, clinical manifestations include cervicitis, urethritis, endometritis, PID, or abscess of the Bartholin glands[7, 5]. Although the initial site of infection is usually the cervix, the urethra and rectum may also be infected. Culture studies have shown that among women infected with *C. trachomatis*, 50 to 60% are infected at both the cervix and urethra, 30% have only cervical infections, and 5 to 30% have only urethral infections[8]. Pelvic Inflammatory Disease, which results from ascending infection, is responsible for most of the morbidity and cost resulting from chlamydial infection[9]. Chlamydial infections can apparently cause more severe tubal immunopathology than other agents in spite of the absence of overt symptoms. This is most probably due to the greater chronicity and fulminating character of Chlamydial infections compared with more acute infections such as gonorrhea[10]. Silent and untreated salpingitis is now recognized as a major cause of infertility as more than 50% of women with documented tubal occlusion report no history of PID but show serologic evidence of previous *C. trachomatis* infection. Similarly, multiple studies have shown associations between previous Chlamydial infection, both symptomatic and asymptomatic, and ectopic pregnancy. The prevalence of *C. trachomatis* infection in pregnant women ranges from 2% to 35%[11]. Pregnant women with Chlamydial infections are at increased risk for adverse outcomes of pregnancy, and postpartum PID. In one study by Martin *et al*.,[12] pregnant women with *C. trachomatis* infection were 10-fold more likely to have outcomes of stillbirth and neonatal death. Gestation periods were also significantly shorter in infected women.

There are 70 different strains of *N. gonorrhoeae*. Gonorrhea, or infection with the gram-negative coccus *Neisseria gonorrhoeae*, is a major cause of morbidity among sexually-active individuals worldwide. Extragenital infections of the pharynx and rectum are prevalent in certain groups, such as men who have sex with men (MSM). Invasive infections with *N. gonorrhoeae*, including disseminated gonococcal infection, endocarditis, and meningitis, are uncommon but can result in serious morbidity[13,14]. Gonococcal resistance to several classes of antimicrobial agents is widespread. The growing threat of antimicrobial resistance in *N. gonorrhoeae* highlights the importance of ensuring the availability of appropriate diagnostic modalities for surveillance[15]. *Neisseria gonorrhoeae* is a common cause of urethritis, particularly in urban areas. Disproportionately higher rates of infection are reported from sexually transmitted infection (STI) clinics compared with private sector settings, in part due to diagnostic and reporting characteristics in public settings. Sexually Transmitted Infection clinic-based studies suggest that the majority of infected men are symptomatic[16]. In contrast, population-based studies, suggest that up to 60 percent of men may be asymptomatic or have very mild symptoms. Two methods for detecting *N. gonorrhoeae* are culture and non-culture tests. Culture techniques are considered the tests of choice; but non culture techniques, which are less labor-intensive and are similar in accuracy to cultures, have replaced culture techniques in some instances. The newest non culture technique is the nucleic acid amplification test[17]. This test has good sensitivity (92 to 96 percent) and specificity (94 to 99 percent) compared with cultures.

As aforementioned, gonorrhea is frequently asymptomatic, and if symptoms are present, they are commonly nonspecific. Accordingly, appropriate laboratory diagnostics are crucial for confirmed diagnosis, case finding, and test of cure. This study aimed at evaluating *C. trachomatis* and *N. gonorrhoeae* infections and their co-infection among women with infertility issues at gyneacological clinics in Ado–Ekiti, establish the level of co-infection of *C. trachomatis* and *N. gonorrheae* among patients attending gyneacological clinics in Ado Ekiti L.G.A., Ekiti State, investigate the correlation between *N. gonorrhoeae* and *C. trachomatis* and infertility, compare the diagnostic methods, using the results obtained from both the rapid test kit results and PCR results and confounding factors in the diagnosis of the two infections. *N. gonorrhoeae* PID is often mistaken as *C. trachomatis* and both have been established to be responsible for high rate of infertility in both male and female. These organisms caused both preventable and treatable infertility and adverse pregnancy outcome, even though they can be asymptomatic and silent in some patients[18,19].

MATERIALS AND METHODS

Study Area

The study area is Ado–Ekiti, headquarter of Ado Local government area of Ekiti State. It is situated in southwest Nigeria and it is the State capital of Ekiti State. The latitude of Ado–Ekiti 7.612426 and the longitude is

5.237109. Ado–Ekiti is a city with GPS coordinates of 7^0 36'44.7336'N and 5^0 14'13.5924'E. The elevation of Ado–Ekiti is 430.582, with its time zone as Africa/Lagos. The population in 2006 was 308,621. The people of Ado–Ekiti are mainly of Ekiti sub–ethnic group of the Yoruba.

Study Location

This study of *Chlamydia trachomatis* and *Neisseria gonorrhea* co-infection was carried out among patients attending selected infertility clinics within Ado–Ekiti, Ekiti State. The selected hospitals are:

i. Ekiti State Teaching hospital, Ado-Ekiti.
ii. Olive Hospital, along housing road, Ado–Ekiti
iii. Maternal and Child hospital, along Federal Polytechnic/immigration road, Ado–Ekiti.
iv. ABUAD Multi-System Hospital, Ado–Ekiti.

These clinics were selected for their renowned facility, capability and efficiency in handling infertility or gyneacological cases, these clinics has an average of twenty patients or more per clinic days and have consultation days more than once a week.

Study Population

All the women attending infertility clinics within Ado –Ekiti Local Government Area were recruited and evaluated in this study.

Study Design

The study is a cross sectional investigation of infertile women for presence of *C. trachomatis* and *N. gonorrhoeae*. Random sampling of the study population was done using informed consent form.

Sample size

The sample size was obtained using the formula:

$$N = \frac{[(Z_1 - \alpha/2)^2 P(1-P)]}{d^2}$$ [37]

Where N=Minimum sample size

$Z_1 - \alpha/2$ = standard normal variant at 5% type 1 error, $P < 0.05$ =1.96 on the Z table

P = Expected prevalence in population based on previous studies or pilot studies (18.3%) [20].

d = Absolute error or precision, placed at absolute error of 5% = 0.05.

Substituting into the above stated formula:

The number of samples screened for both *C. trachomatis* and *N. gonorrhoeae* infections were 230 (115 urine samples and 115 HVS samples).

Ethical Clearance

Ethical approvals were obtained from the Ethical and Research Committees of both ABUAD Multi-System Hospital, Ado-Ekiti and College of Medicine and Health Sciences, ABUAD after consideration and review of the protocol for this study. The approval identification numbers were AMSH/ REC/EAH/44 and ABUDHREC/10/01/2021/001 from the multisystem hospital and the University respectively.

Sample collection and transportation

Informed consent forms were filled by the participants to ascertain their willingness and voluntary participation. Those who were not comfortable with the exercise were excluded from the study. Questionnaires

were administered to volunteers who filled and were willing to give samples. A total number of 230 samples (urine and HVS samples) were collected from volunteers. It was collected within a period of one year. About 15 mls urine samples in sterile universal bottle with laboratory number, date and time of collection, were packed in a clean leak proof ziplock and transported in ice packs to Medical Laboratory Science (MLS) Department, ABUAD. The HVS tips were cut with a sterile scissors in a viral transport medium (VTM) and transported to MLS Department, ABUAD.

Specimen Processing

Ten (10) mls of urine samples were centrifuged at 1000 rpm for 10 minutes and the supernatants was discarded and the precipitate was used for the detection. 5 mls of the urine samples in a sterile plain sample bottles were frozen at -20^{0}C in the laboratory refrigerator. The urine samples were processed at the laboratory of each selected clinics before transporting it to Medical Laboratory Science department, ABUAD. The HVS tip was also frozen at -20^{0}C in the VTM vials. The frozen samples were transported while maintaining its cold chain to Biorepository and Clinical Virology Laboratory, University College Hospital, Ibadan, Oyo State for PCR test.

Detection of *Chlamydia trachomatis* Antigen in Urine Samples

The rapid test for detecting *Chlamydia trachomatis* antigen was used (Wandfo Biotech Co. Limited catalog No: N 35-C (4.0) mm, South China University of Technology, Guuagyzhon, P.R. China)[39,21]. The Wandfo Biotech one step Chlamydia swab/urine test is a rapid immune chromatographic test for the visual detection of *Chlamydia trachomatis* antigen in urine specimens or endocervical swab specimens. This test adopts double antibodies sandwich method. When exactly four drops of the specimen is added to the sample well, the specimen is absorbed into the device by capillary action, mixes with the antibody-dye conjugate, and flow across the pre coated membrane. When the *Chlamydia trachomatis* antigen levels are at or above the target cut off, the antigen in the specimen binds to the antibody- dye conjugate are captured by monoclonal antibody immobilized in the region (T) of the device. This produces a coloured test and indicates a positive result. When the cut off antigen levels are zero or below the target cut off (the detection sensitivity of the test), there is not a visible colored band in the test region (T) of the dense, which indicates a negative result. To serve as a procedure control, a coloured line will appear at the control region (C), if the test has been performed properly[21].

Test procedure I

Samples were brought to the laboratory, the same day it was collected in a cold chain and were tested immediately without delay. About 15 mls of urine specimen each was collected from volunteers into a clean sterile universal bottle. 10 mls of the urine specimen each was transferred into a clean, dry, sterile centrifuged tubes and was centrifuged at 1000 rpm for 10 minutes. After centrifuging, the supernatant was discarded and the precipitate was used for detection. In the tubes with the precipitated, 300 µl extraction of buffer A was added to

each and mixed well. After five minutes, 300 µl extraction of buffer B was also added to each tube and was mixed. The test cassette was removed from its sealed foil pouch by tearing along the notch. 4 drops (100 µl of the Liquid from the extraction tube) was dispense into sample well of test cassette using a separator dropper for each tubes. The reactions were observed and the results were read and recorded after 5 minutes respectively.

Interpretation of Results

Positive result

Appearance of visible two rose pink bands in both the control region and test region indicates that there is presence of *Chlamydia trachomatis*.

Negative result

One rose-pink band in the control region with no apparent band in the control region with no apparent band in the test region indicates that *Chlamydia trachomatis* antigen is absent

Detection of *Neisseria gonorrhoeae* Antigen in Urine Samples

The rapid test kit for *N. gonorrhoeae* manufactured by Maternova Biotech Co. Catalog No: MA 2033, North America, USA. The test works by defecting the *N. gonorrhoeae* antigen in the urine/urethral sample as it flows through the assay by first; *N. gonorrhoeae* antibodies conjugated with colloidal gold that attach to *N. gonorrhoeae* antigen and provide a "visual tag" for antigen – antibody colloidal gold complex and second; by a last zone with immobilized antibodies that capture the complex, the visual tags producing a visible pink-coloured band that is confirmation of the presence of *N. gonorrhoeae* and a positive result for gonorrhea. If there is no pink line, *N. gonorrhoeae* is not present, indicating result for absence of gonorrhea. Results are ready in few minutes[22].

Test procedure II

The cassette was removed from the seal pouch and was used immediately. The extraction buffer was added to the urine deposit, mixed properly and left for about 2 minutes. The test cassette was placed on a clean and leveled surface, 3 drops of the extraction solution (approximately 100 ml) was dropped into the specimen into the specimen well of the test cassette, then the start timer was set. It was observed for coloured line(s). It was read after 10 minutes.

Interpretation of Results

Positive result

Appearance of visible two pinks bands in both the control and test regions indicates that there is presence of *N. gonorrhea*

Negative result

One pink hand in the control region with no visible band at the test region indicates that no *N. gonorrhea* antigen in present.

Invalid result

Either one pink band or two pink band in control region only or both control and test region are considered invalid if cassette are left unread after 30 minutes and still showed visible bands

Polymerase Chain Reaction (PCR)

DNA was extracted from 200 µl urine samples under sterile conditions using DaAnGene extraction kit (decode genetics)

following the manufacturers manual. The extracted DNA (s) was stored at –20ºC until amplification.

qPCR primer design

The 23S rRNA genomics DNA specie specific region was used to design specific primers for the diagnosis of *C. trachomatis* and *N. gonorrhoeae*. 23S rRNA sequences for *C. trachomatis* were obtained from the genebank database on National Center for Biotechnology Information (NCBI) website. This was done by accessing NCBI website (*http://www.ncbi.nlm.nih.gov/*), then selecting nucleotide from the drop down and key in *C. trachomatis* 23S rRNA gene in the search box and send, sequence related were made accessible and a total of 50 accessions were selected for cluster multiple alignment. These sequences were uploaded in a bioedit software and cluster ran to reveal converse regions unique to all strains of *C. trachomatis* 23S rRNA and this was used in primer designing. *https://www.idtdna.com/PrimerQuest/Home/Index* site was then accessed and sequenced pasted in the sequence entry box and multiple qpcr primers were generated. It is very necessary to ensure that the primers anneal to region where *C. trachomatis* 23S rRNA sequences are conserved. Each primer pair was then checked for specificity to be sensitive to only *C. trachomatis* 23S rRNA and also ability to cut across all strains then the best primer was selected and synthesized in Inqaba Biotech in South Africa. The 23S rRNA genomic DNA for *N. gonorrhoeae* gene was also designed following the same procedure.

qPCR

qPCR was carried out using the Luna universal qpcr master mix (New Biolab) following the manufacturers protocol. 20 µl total reaction volume consisting of 10 µl Luna universal qPCR master was mix, 0.25 µl forward and reverse primer were made from 15µl with sterile distilled water to which 5 µl DNA template was added. qPCR reaction was carried out in a CFX96 real time system C1000 thermal cycler system (BioRad., USA) with a cyclic conditions including initial denaturation at 95ºC for 1 minute, followed by 40 cycles of each cycle comprised of 30 seconds denaturation at 95ºC, 30 seconds annealing at 60ºC, plus plate reading, termination at 72ºC for 10 minutes. Results were read on the screen based on the threshold cycle (CT Value) of each cycle using each primer.

Data Analysis

Results obtained from Laboratory tests kit, questionnaires and qPCR test results were summarized on tables with frequency and percentage.

Table 1: Showing the forward and reverse primer sequence

Specimen	Primer name	Primer sequence
C. trachomatis	ChTF	TGATAGCGTCACACCAAGTG
	ChTR	CCTGCTGAACCAAGCCTTAT
N. gonorrhoeae	NeGF	CGTAGACACTCGTCATCACTTC
	NeGR	TAGCTTGTTTAAGCCGGTAGG

RESULTS

Demographic Characteristics of the Subjects

The age of participants ranges between 25–50 years, the highest percentage (34.7%) of the participants in this study fell within the age range of 36–40 years while the lowest percentage (9.6%) of participant were in the age range of 20–25 years (Table 2). A total of 98.2% of the participant were currently married while the rest were either divorced (0.9%) or have re–married (0.9%), (Table 2). The highest percentages (34.8%) of the participants have been married for 7–10 years while 1.7% of them were in marriage of less than one year (Table 2). A total of 69.6% of the participant have had previous history of sexually transmitted disease while 30.4 had no such record (Table 2). In the present study, about 56% of the participants have combination of signs and symptoms of sexually transmitted infections. About 23% reported no such symptoms while 10.4% reported itching as most obvious sign of STI (Table 2). A percentage of 47 of the participants reported improper diagnosis of their condition at their hospitals while 61% admitted that they were properly diagnosed (Table 2). There were various responses as to the spouse of the participant agreeing to be treated for the symptoms noticed (Table 2). About ninety percent (91.3%) of the participant admitted that they have been not been pregnant before (primary infertility) while 8.7% were cases of secondary infertility (Table 2). About 4.3% of the participant recorded miscarriage at 1–2 months, 3.55 had miscarriage at 3–4 months of pregnancy, 0.9% confirmed miscarriage at 5–6 months while about 91% had no experience of miscarriage (Table 2). Some of the participants (8.7%) have had previous cases of fibroid while about 90% never had a case of fibroid. History of stillbirth was reported by 7.0% of the participant and over ninety (93%) had no such experience (Table 2).About 5 % were not aware if they had had any form of hormonal imbalance as they were never tested nor diagnosed for it, some (7%) agreed of being diagnosed, while 89.6% were never tested or diagnosed of any form of hormonal imbalance (Table 2). About 55.6% of the participant admitted to have had some STI/STD's symptoms at different time but never treated and felt these symptoms has cleared without any treatment, 2% are indifferent as they do not treat nor felt any symptoms, 42.6% agreed to have treated in different ways (Table 2). Among the participants, 4.3% admitted that there was a delay in childbearing in their family and that of their spouses, about 8% had no idea of any delay in either their family or that of their spouses', over 80% reported there was no such history in both their family and the spouses' family (Table 2). About 3.5% admitted that for a period of 4–6 years there was delay in child bearing in their family lineage, 4.3% reported 7–9 years, 11.3% agreed it was over 10 years, 80.9% reported no such waiting period in their family lineage (Table 2).

Analysis of the Samples by the Diagnostic Methods Employed

When urine samples were tested with rapid kit, 31.3% tested positive to *C. trachomatis* and 33.9% tested positive to *N. gonorrhoeae* (Table 3). With qPCR, 32.3% tested positive to *C. trachomatis* when urine samples were tested and 42.6% tested positive to *N.*

gonorrhoeae with urine samples as well (Table 4). In the current study, HVS samples tested for *C. trachomatis* and *N. gonorrhoeae* infections with qPCR, 11.3% tested positive to *C. trachomatis* and 44.35 tested positive to *N. gonorrhoeae* (Table 5). In the study, a co–infection of these microorganisms was evaluated with rapid test kit and qPCR, in urine samples from these subjects, 16.5% and 20.0% tested positive with rapid kit and qPCR respectively from urine (Table 6). HVS tested with qPCR recorded co–infection rate of 7.8% (Table 6). The co–infection in urine samples tested with rapid test and co–infection in urine sample tested with qPCR, were not significantly different (P>0.05) (Table 7). The level of co–infection in urine sample tested with rapid kit and co–infection from HVS tested with qPCR is significantly different (P<0.05) (Table 7). The number of positive cases for *C. trachomatis* in HVS tested with qPCR was significantly different from that of urine tested with rapid kit (P < 0.000) (Table 8). Table 9 showed significance differences in the number of participant who tested positive *N. gonorrhoeae* when HVS was tested with qPCR and urine rapid test (P<0.05). HVS samples tested with qPCR for *N. gonorrhoeae* infection and urine samples tested with qPCR for *N. gonorrhoeae* as well were not significantly different (P>0.05) (Table 10) while urine samples tested with qPCR for *C. trachomatis* and HVS tested with qPCR for this same organism were significant (P<0.05) (Table 11). The level of co–infections of HVS tested with qPCR and that of urine tested with qPCR were also significant (P<0.05) (Table 12). The confounding factors such as; previous history of fibroid, properly treated infections either with antibiotics or injections, those that have experienced still birth or ectopic pregnancy, subjects with previous history of delay or bareness in either their family or husband's. This study showed that these factors were insignificant to the outcome of the test results.

Table 2: Demographic characteristics of subjects from the selected hospitals with frequency and percentage

Age distribution of participants	N	%
20 – 25	11	9.6
26 – 30	18	15.7
31 – 35	30	26.1
36 – 40	40	34.7
41 – 50	16	13.9
Total	**115**	**100.0**
Marital status	N	%
Currently married	113	98.2
Divorced	1	0.9
Re – married	1	0.9
Total	**115**	**100.0**
Duration the participants have been married		
Number of year(s) married	**N**	**%**
>1	2	1.7
1 – 3	25	21.7
4 – 7	27	23.5
7 – 10	40	34.8
> 10	21	18.3
Total	**115**	**100.0**
Previous history of sexually transmitted infection or diseases		
Responses	**N**	**%**
Yes	80	69.6
No	35	30.4
Total	**115**	**100.0**
Visible signs and symptoms noticed by participants for STI/STDs		
	N	%
None	27	23.5
Itching	12	10.4
Smelling discharge	5	10.4
Painful urination	1	0.9
Delayed menstrual cycle	2	1.7
Smelly menstrual cycle	3	2.6
Combination of symptoms	65	56.5
Total	**115**	**100.0**
Improper diagnosis of subjects' at their various hospitals		
Response	**N**	**%**
Yes	54	47.0
No	61	53.0
Total	**115**	**100.0**
Response to the treatment of previous infection by the subjects' spouses		
Response	**N**	**%**
Not applicable	12	10.4
Strongly agree	23	20.0
Agree	19	16.5
Neutral	2	1.7
Strongly disagree	21	18.3
Disagree	38	33.0

Total	**115**	**100.0**
Number of subjects that have been pregnant before		
Response	**N**	**%**
Yes	10	8.7
No	105	91.3
Total	**115**	**100.0**
Duration of the pregnancy had by the subjects before miscarriage		
	N	**%**
Not applicable	105	91.3
1 – 2 months	5	4.3
3 – 4 months	4	3.5
5 – 6 months	1	0.9
	115	**100.0**
Previous history of fibroid		
Response	**N**	**%**
Yes	10	8.7
No	105	91.3
Total	**115**	**100.0**
Previous history of still birth or ectopic pregnancy among subjects' tested		
Response	**N**	**%**
Yes	8	7.0
No	107	93.0
Total	**115**	**100.0**
Number that were diagnosed with hormonal imbalance		
Response	**N**	**%**
Yes	7	6.1
No	103	89.6
Not aware	5	4.3
Total	**115**	**100.0**
Number of subject that had signs / symptoms of STI/STDs and never treated with spouses but felt it has cleared after a while.		
Response	**N**	**%**
Not treated	64	55.6
Indifferent	2	1.7
Treated	49	42.6
Total	**115**	**100.0**
History of delay in childbearing in the subjects' family or spouses'		
Response	**N**	**%**
Yes	5	4.3
No	100	87.0
Not aware	10	8.6
Total	**115**	**100.0**
History of delay and duration of waiting before childbirth in the subjects' or spouses' family		
Number of years	**N**	**%**
Not applicable	93	80.9
4 – 6 years	4	3.5
7 – 9 years	5	4.3
>10 years	13	11.3
Total	**115**	**100.0**

Table 3: Numbers of positive cases of the microorganisms tested with rapid test kits using urine samples (n = 115).

Parameters	Positive	Negative	Percentage positive (%)
Number of *C. trachomatis* that were positive in urine samples tested with rapid kit.	36	79	31.3
Number of *N. gonorrhoeae* that were positive in urine samples tested with rapid kit.	39	76	33.9

Table 4: Numbers of positive cases of the microorganisms tested with qPCR using urine samples (n = 115).

Parameters	Positive	Negative	Percentage positive (%)
Number of *C. trachomatis* that were positive in urine samples tested with rapid kit.	37	78	32.2
Number of *N. gonorrhoeae* that were positive in urine samples tested with rapid kit.	49	66	42.6

Table 5: Numbers of positive cases with qPCR using high vaginal swab (HVS) samples (n = 115).

Parameters	Positive	Negative	Percentage positive (%)
Number of *C. trachomatis* positive cases when HVS samples were tested with qPCR.	13	102	11.3
Number of *N. gonorrhoeae* positive cases when HVS samples were tested with qPCR.	51	64	44.3

Table 6: Level of co – infection by the microorganisms (n = 115).

Parameters	Positive	Negative	Percentage positive (%)
Level of co – infection using urine when tested with rapid kit.	19	96	16.5
Level of co – infection using urine when tested with qPCR.	23	92	20.0
Level of co – infection using HVS when tested with qPCR.	9	106	7.8

Table 7: Rate of co–infection of both microorganisms tested with rapid kit and qPCR.

Parameters	Number positive	T – value (t ≥ 1.98)	P – value (< 0.05)
Co – infection in urine samples tested with rapid kit.	19 (11.5)	- 1.138	0. 259
Co – infection in Urine sample tested with qPCR.	23(20)		
Co- infection in urine sample tested with rapid kit.	19 (11.5)	2.095	0.038
Co – infection from HVS tested with qPCR.	9 (7.8)		

Table 8: Number of *C. trachomatis* positive in urine tested with rapid test and HVS tested with qPCR.

Parameters	Number positive	T – value (t ≥ 1.98)	P- value (<0.05)
HVS using qPCR.	13 (11.3)	-4.599	0.000
Urine using rapid test.	36 (31.3)		

Table 9: Number of *N. gonorrhoeae* positive in HVS tested with qPCR and urine tested with rapid test.

Parameters	Number positive	T – value (t ≥ 1.98)	P – value (<0.05)
HVS tested with qPCR.	51 (44.3)	-0.470	0.000
Urine tested with rapid test.	39 (33.9)		

Table 10: Number of *C. trachomatis* positive when both urine and HVS were tested with qPCR.

Parameters	Number positive	T – value (t ≥ 1.98)	P – value (<0.05)
Urine tested with qPCR.	37 (32.2)	0.428	0.000
HVS tested with qPCR.	13(11.3)		

Table 11: Number of *N. gonorrhoeae* positive when both urine and HVS were tested with qPCR.

Parameters	Number positive	T – value (t ≥ 1.98)	P – value (<0.05)
Urine tested with qPCR.	49 (42.6)	7.368	0.639
HVS tested with qPCR.	51 (44.3)		

Table 12: Number of co – infection positive when both urine and HVS were tested with qPCR.

Parameters	Number positive	T – value (t ≥ 1.98)	P – value (<0.05)
Co – infection urine tested with qPCR.	23 (20)	0.577	0.000
Co – infection HVS tested with qPCR.	9(7.8)		

DISCUSSION

C. trachomatis and *N. gonorrhoeae* have long been known to cause mild to chronic infection in humans such as urethritis, endemic trachoma, cervical pains, pelvic inflammatory disease, painful urination, ectopic pregnancy amongst others. This study was aimed at evaluating the level of *C. trachomatis* and *N. gonorrhoeae* infections as well as determining the level of co–infection of these microorganisms among women attending infertility clinics within Ado–Ekiti. Statistical data obtained from Nigeria (Southern, South East, Northern parts), Ghana, and other developing countries such as Philippines, Asia and India revealed that the screening for either or both organisms is not included in the routine/regular test in either antenatal or any gyneacological/fertility clinics probably because of their silent nature or due to lack of possible publicity, lack of information on proper screening methods of these microorganisms, and their mis-diagnosis. These organisms have been implicated as cause of health problems in both males and females, pregnant women, newborns as well as the foetus. In this study, emphasis was on each of the organisms as causes of severe reproductive damage individually and in combination because of the way both organisms are mistaken for each other considering the similarity in their clinical presentations, although differing in the treatment approach due to the hierarchy of drug usage. In the studied population, a total of 230 specimens were tested: 115 urine samples were tested for both *C. trachomatis* and *N. gonorrhoeae*, 115 HVS specimen were tested for these same organisms. Both samples were collected from same subjects. *C. trachomatis* infection is most commonly reported natural noticeable disease in the United States, with more than 900,000 cases reported to state and local health departments in 2015, among obstetrics and gyneacology cases. So also in Philippines, between the year 2012–2016, with about 53% positive among sex female workers and 62% among already confirmed cases of infertility[23] and in countries like United State, Philippines, indiscriminate use of antibiotics is not common hence the validity of the reports recorded. This report from Philippines is higher than the results from Chlamydia screening from the present study. This is likely to be due to the category of subjects screened but still confirming the existence of this organism in females. The prevalence of genital chlamydia and gonococcal infections in women at risk in the Kumasi Metropolis, Ghana, and between women aged between 18–35 years with a history of having at least 3 sexual acts per week and having at least 2 sex partners in the previous 3 months reported 4.8% positive to chlamydia and 0.9% participants positive to gonococcal infection[24]. A study done on women who attended the gyneacology clinic of Korle Bu Teaching Hospital, Accra, Ghana for various reasons reported a prevalence of 4.9% and 3.1% respectively for *C. trachomatis* and *N. gonorrhoeae* In the same study, postpartum women tested for these organisms showed 7.7% and 3.4% positive to *C. trachomatis* and *N. gonorrhoeae*[25]. Dela *et al.*[26] found gonorrhoeae and chlamydia transmission in selected health facilities in Ghana as 28% who had gonorrhoeae and 11% who had

chlamydia. The reported positive results were low to that obtained from this study. Understanding the incidence rate and the multinational epidemiology of *N. gonorrhoeae* has helped developed countries with prevention and control policies. The World Health Organisation estimated that approximately 87 million new Gonococcal infections occurred among 15–49 years old in 2016[27]. The estimated global prevalence in 2012 was 13 million cases of *C. trachomatis* and 78 million cases of *N. gonorrhoeae* in adults between the ages of 15-49 years[28]. The annual estimation indicates different rates from 1.9%-30.6% in pregnant women and 1.6–18% in neonates[29]. It was also reported that *C. trachomatis* is the most frequent infectious agent accounting for 18% to 50% of all neonatal conjunctivitis and 3% to 20% of infantile pneumoniae[29]. Likewise, about half of the neonates born from infected mothers with *N. gonorrhoeae* will develop neonatal conjunctivitis[30]. Untreated gonococcal conjunctivitis may lead to corneal scarring and blindness whereas the risk of severe ocular damage is high with a co–infection with *C. trachomatis*. A prevalence of 38.18% was reported by Costumbrado *et al*[31]. This prevalence of *C. trachomatis* infections in patients attending gyneacological clinics was reported in Zaria, Northern Nigeria. High vaginal swab samples were used and enzymes linked immunosorbent assay (ELISA) was used for the detection. This prevalence was higher than the result obtained from this research (11.3%), probably due to the different method used in screening. In 2016, Okunola *et al*[32] carried out this study on antenatal patients in Ile–Ife, to find out the prevalence of *Chlamydia trachomatis* only. It was recorded that 10%-15% of untreated Chlamydia cases led to pre–term delivery and neonatal conjunctivitis after a follow up with the antenatal patients for a period of time. Adachi *et al*[33] reported a research on *C. trachomatis* on sexually active asymptomatic females in Okada, South–South, Nigeria using different types of kits to screen 170 females using their urine samples a total of 7.85% were found positive despite their asymptomatic state. The result (32.2%) from this research is higher which may be due to the category of subjects tested. However, this previous research has shown even if a patient is asymptomatic, the 'silent' Chlamydia is present. Keshinro, *et al*[20], studied the prevalence of HIV, Chlamydia and Gonorrhoeae co–infections among men having sex with men, and transgender women in Abuja and Lagos State, Nigeria. A prevalence of 18.3% co-infection of *N. gonorrhoeae* and *C. trachomatis* was reported among women in Lagos State. This is lower to the result (20%) recorded in this research, but this has also confirmed the possibility of co–infection of these microorganisms among women. These are major health concerns in which this research has helped to shed more light. Consequently, if the mothers are checked for these organisms, proper treatment and monitoring will be given to both waiting and pregnant women, so as to avert any complications that can arise. This study has also showed that the prevalence of *N. gonorrhoeae* is higher when urine samples were tested with both rapid kit and qPCR as well as when HVS

samples were tested with qPCR, hence the organism is of medical importance. Also, based on this study, *C. trachomatis* urine sample is better for its diagnosis compared to high vagina swab.

CONCLUSION

Comparative methods used in screening for *C. trachomatis* and *N. gonorrhoeae* in the research work have clearly shown that polymerase chain reaction test procedure is significant. It revealed the presence of these microorganisms in samples analyzed. Despite this, rapid test kit for preliminary screening is also significant. Most previous studies were basically done using complement fixation test, rapid test kit and ELISA. The most recommended is the use of PCR, despite its drawback in cost of running this test, is still worth screening patients especially those attending infertility clinics and the best sample to use when testing for *C. trachomatis* is urine while for *N. gonorrhoeae*, high vagina swab (HVS).

RECOMMENDATION

Proper screening of patients: women attending infertility clinics, pregnant women, and their husbands for *C. trachomatis* and *N. gonorrhoeae* as a routine test before undergoing IVF and /or delivery. Proper and consistent monitoring and a repeat test should be carried out after a month from the first time of being tested and treated. Patients, health providers should be educated at all level of health care.

Acknowledgement

The authors would like to acknowledge the staff of the Department of Medical Laboratory Science, Afe Babalola University for their contribution during the study.

Conflict of interest: Authors have no conflict of interest to declare

REFERENCES

1. World Health Organization, (WHO). (2015). Global incidence and prevalence of selected curable sexually transmitted infections - 2008. *http://www.who.int/reproductive health/publications/rtis/stisestimates/ en/*. Accessed on February 18, 2022.
2. World Health Organization (WHO). (2018). WHO report on global Sexually Transmitted Infection Surveillance. *Wind Heal Organ. Communicable Disease Intellect Q* Reports; 34(1): 1- 7.
3. Hossam, A. A., Jan P. and Gerry B. (2013). Genetic variation in *Chlamydia trachomatis* and their host: Impact on disease severity and tissue tropism. *Indian Journal of Pathology Microbiology*; 15(6): 78 – 84.
4. Center for Disease Control and Prevention, (CDC). (2019). Compendium of measures and control. *Chlamydia psittaci* infection array humans (psittacosis) and pet birds (Avian chlamydiosis).
5. Arlieke, G., Niek, N. S. and Daisy V. (2019). Chlamydia infection from outside to inside. *Frontier Microbiology*. 10, 2329.

https://doi.org/10.3389/fmicb.2019.02329.

6. Charlotte, A. J., James, H., Nicholas, R. T. and Collette, O' N. (2020). The Nature and Extent of Plasmid variation in *Chlamydia trachomatis*; 30: 2 – 6.
7. Soper, D. E. (2010). Pelvic inflammatory disease. *Journal of Obstetrics and Gynaecology*; 116: 419– 428.
8. Deniz D., Mahnut C., Ekin E., Demir S., Remziye T. and Ece T. (2020). Chlamydia infection in infants. Clinical Bacteriology and Infectious Disease. Journal of Tropical Pediatrics; 12: 233 – 236.
9. Abbai, N. S., Wand, H. and Ramjee, G. (2013). Sexually transmitted infections in women participating in a biomedical intervention trial in Durban: Prevalence, co-infection and risk factor. Journal of Sexually Transmitted Disease; 20: 2 – 8.
10. Helena, D., Naiki, A., Eric, B., Selassie, K., Kwasi, K. A., Edward, O. N., Nicholas, N.A., Kyei, J. and Nii, A. (2019). Risk factors associated with Gonorrhoeae and Chlamydia transmission in selected health facilities in Ghana. BioMed Central Infectious Disease; 19: 425 - 427.
11. Kalpana B., Jamie, M. R., Gong T. and Catherine, L. H. (2016). Prevalence of Chlamydia trachomatis among child bearing age women in India: A systematic review. Journal of Infectious Diseases in Obstetrics and Gyneacology; 2016: 1 – 6.
12. Martin, D. H. (2015). The microbiota of the vagina and its influence on women's health and disease. American Journal of Medical Science; 343: 2 – 9.
13. Janda, W. M. and Gaydos, C. A. (2007). Neisseria. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry & M. A. Pfaller (Eds.), Manual of Clinical Microbiology. 9th edition. Washington, D.C.: ASM Press. Pp. 601-620.
14. Chernsky, M. A., Lee, H., Jary, D., Luistra, K., Chong, S., Sellors, L., Pikard, L. and Mahony, N. (2015). Female Chlamydia trachomatis genitourinary infections diagnosed by testing cervical swabs and first void urine by Polymerase chain reaction and Ligase chain reaction assays. Abstract, D105. Pp. 85. In program and abstract of the 35th interscience conference on antimicrobial agents and chemotherapy. American Society of Microbiology, Washington D.C.
15. Ison, C. A. (2016). Neisseria gonorrhoeae. In S. H. Gillespie, & P. M. Hawkey (Editors). Principles and Practice of Clinical Bacteriology. 2nd edition. England: John Wiley & Sons Limited. Pp. 221-230.
16. Workowski, K. A., Berman, S. M. and Douglas, J. M. (2018). Emerging antimicrobial resistance in Neisseria gonorrhoeae: urgent need to strengthen prevention strategies. Annals of Internal Medicine; 148(8): 606 - 613.

17. Vickerman, P., Peeling, R. W., Watts, C. and Mabey, D. (2015). Detection of gonococcal infection: pros and cons of a rapid test. Molecular Diagnosis; 9(4): 175-179.
18. World Health Organization. (2011). Global prevalence and incidence of selected curable sexually transmitted diseases: Overview and estimates. Africa Region.
19. Lai-king, N. G. and Irene, E. M. (2015). The laboratory diagnosis of N. Gonorrhoeae. Journal of Infectious Diseases and Medical Microbiology; 16(1): 15 - 25.
20. Kenshinro, B., Crowell, T. A, Nowak, R. G., Adebayo, S., Peel, S., Gaydes, C.A., Rodriguez – Hart, C., Baral, S. D. … and Ake, J. (2016). High prevalence of HIV, Chlamydia and Gonorrhoeae among men who have sex with men and transgender women attending trusted community centers in Abuja and Lagos, Nigeria. Journal of International AID Society. 7; 19(1): 21 - 27.
21. Wondfo biotech, (2021). Chlamydia trachomatis antigen (Wandfo Biotech Co. Limited catalog No: N 35-C (4.0) mm, South China University of Technology, Guuagyzhon, P.R. China).
22. Maternova Biotech. (2022). Neisseria gonorrhoeae manufactured by Maternova Biotech Co. Catalog No: MA 2033, North America, USA
23. Center for Disease Control and Prevention. (CDC). (2016). Sexually transmitted disease treatment guidelines, morbidity and mortality weekly Report; 55 (RR - 11): 38 - 42.
24. Opoku, B. K. and Sarkodie, Y. A. (2010). Prevalence of genital Chlamydia and Gonococcal Infections in at risk women in the Kumasi Metropolis, Ghana. Ghana Medical Journal; 44(1): 21 – 24.
25. Bentsi, C., Klufio, C. A., Perine, P. L., Bell, T. A., Cles, L. D., Koester, C. M., Wang, S. P. (2015). Genital infections with Chlamydia trachomatis and Neisseria gonorrhoeae in Ghanaian women. Genitourin Medicine. Journal of Sexually Transmitted Disease; 51(2): 48 – 50.
26. Dela, H., Attram, N., Behene, E., Kumordjie, S., Addo, K.K., Nyarko, O., Kyei, N. N. and Carrol, J. N. (2019). Risk factors associated with gonorrhoeae and chlamydia transmission in selected health facilities in Ghana. Biomed Center for Infectious Disease; 19: 425.
27. Robert, D. K., Weston, E., Aluisio, C. S. and Hughes, G. (2019). Epidemiology of Gonorrhoeae: A global perspective. Sex health; 16 (5): 401 - 411.
28. Newman, L., Rowley, J., Vander Hoorn, S., Wijesooriya, N. S., Unemo, M., Low, N., Stevens, G., Gottlieb, S., Kiarie, J., & Temmerman, M. (2015). Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based onSystematic Review and

Global Reporting. PloS one, 10(12), e0143304.https://doi.org/10.1371/journal.pone.0143304

29. Pourabblas, B. Rezaei, Z., Mardaneh, J., Shahian, M. and Alborzi, A. (2018). Prevalence of Chlamydia trachomatis and Neisseria gonorrhoeae infection among pregnant women and eye colonization of their neonates at birth time, Shiraz, Southern Iran. Biomedical Infectious Disease; 18(477): 3382 – 3384.
30. Mohammed, H. I. D., Jatau, E. D.,Olonitola, O. S., Abudl, M. A., Aliyu, M. S. and Tijani, M. B. (2016). Association of Chlamydia serology with HIV in Nigerian women. Nigerian Journal of Basic and Applied Science; 24(2):15 – 22.
31. Costumbrado J, Ng DK, Ghassemzadeh S (2024). Gonococcal Conjunctivitis. [Updated 2022 Sep 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459289/
32. Okunnola, T. O., Ajenifuja, K. O., Abiodun, A. O. (2016). Prevalence of Antenatal Chlamydia trachomatis infection in Ile – ife, Nigeria. International journal of infection. 10: 177-179.
33. Adachi, K. N., Nielsen-Saines, K., & Klausner, J. D. (2021). Chlamydia trachomatis Screening and Treatment in Pregnancy to Reduce Adverse Pregnancy and Neonatal Outcomes: A Review. Frontiers in public health, 9, 531073. https://doi.org/10.3389/fpubh.2021.53107