Original

Molecular Screening for *Chlamydia trachomatis* among Pregnant Women Attending Ante-Natal Clinics in Kaduna Metropolis, Kaduna State, Nigeria

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Abstract

Background: *Chlamydia trachomatis* infection causes pelvic inflammatory disease and infertility and in pregnant women, it is associated with ectopic pregnancy and abortion. One of the key factors in the prevention of *C. trachomatis* infection is the availability of specific laboratory diagnostics with high sensitivity. For economic reasons, serology is the method routinely used for detection of the bacteria in clinical settings in Nigeria. This study was undertaken to investigate the prevalence of infection with *Chlamydia trachomatis* among pregnant women attending antenatal clinics in Hospitals within Kaduna metropolis using serological and molecular techniques.

Method: A cross-sectional study design was implemented in this study. The study recruited 200 pregnant women. Serology was carried out by ELISA technique to detect chlamydia antibody in the sera of subjects. For molecular technique, Bacterial DNA was isolated from ten sera samples that showed positive results with ELISA and the *omp1* gene of *Chlamydia trachomatis* was amplified with specific primers using conventional PCR.

Results: Of the 200 sera samples tested for *C. trachomatis* by ELISA, 19 (9.5%) samples gave positive results, while only 4 of the 10 samples tested by PCR returned positive for *C. trachomatis*.

Conclusion: This data suggests that PCR method is much more specific compared to the serological approach routinely used in diagnosis of the disease, thereby recommending that nucleic acid testing should be incorporated into the diagnosis of this pathogen in clinics and hospitals in developing countries like Nigeria.

Keywords: *Chlamydia trachomatis*, ELISA, PCR, pregnant women, antenatal clinics, Kaduna metropolis.

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Introduction

Chlamydia trachomatis is an obligate intracellular parasite that replicates within the cytoplasm of host cells. As intracellular bacteria, *C. trachomatis* can exist in resting and infectious forms within human epithelial host cells. Various Chlamydia trachomatis serovars are capable of infecting and thriving in a range of host environments, each characterized by specific tissue preferences. This adaptability leads to the manifestation of a broad array of diseases in humans.[1] *C. trachomatis* is differentiated into 18 serovars on the basis of antibody-based typing assays.[2] *Chlamydia trachomatis* is the most impactful species within the Chlamydia genus when it comes to public health, contributing to a significant burden of disease worldwide. It is responsible for millions of genital tract infections annually, with women being particularly affected.[3] While chlamydia is often an asymptomatic infection in women, it is also an important cause of cervicitis, urethritis, and pelvic inflammatory disease (PID). Infections that spread upward through the genital tract, affecting the endometrium, fallopian tubes, or nearby pelvic structures, can lead to serious complications. These may include an increased risk of infertility, ectopic pregnancies, and persistent pelvic pain.[4–6]

Untreated Chlamydia infections have been linked with adverse pregnancy outcomes, especially for miscarriage, stillbirth, and preterm birth.[3] and conditions in the new born such as ophthalmia neonatorum (conjunctivitis) and pneumonia.[7,8]

The exact process by which *Chlamydia trachomatis* infection contributes to adverse pregnancy outcomes remains unclear. However, it is hypothesized that the bacterium may directly infect the fetus, provoking an inflammatory reaction characterized by cytokine release. This immune response could result in complications such as miscarriage, premature rupture of membranes, or preterm labor. Alternatively, the infection might elicit a maternal inflammatory response due to similarities between chlamydial 60 kiloDaltons (kDa) heat shock proteins and their human counterparts, potentially leading to embryonic rejection.[9] It is further suggested that immune reactions triggered by chlamydial heat shock protein (CHSP-60) could play a role in causing damage to the fallopian tubes. This damage may contribute to conditions such as tubal infertility and ectopic pregnancy.[10]

Chlamydia expresses a major outer membrane protein (MOMP) that is surface-exposed and forms the basis for classification of different serovars of *C. trachomatis*. The *omp1* gene, responsible for encoding the major outer membrane protein (MOMP), is found across all three *Chlamydia* species that infect humans. This gene serves as a key marker for determining the genotypes of *C. trachomatis*. [11]

Various diagnostic methods are available for identifying *Chlamydia trachomatis* infections. Culture of the organism in cycloheximide treated McCoy cell monolayers and demonstration of the glycogen containing characteristic intercellular inclusion remains the best standard for viable chlamydia detection, however it is technically difficult, expensive and the need of endocervical swabs to obtain the live organism is another limitation of the culture method.[12,13]

Serological tests based on the immunological detection and typing of the major outer membrane protein (MOMP)[14] or determination of raised titre of anti-chlamydia antibodies usually in the form of immunoglobulin G[15] or IgG and IgM[16] can be performed to detect immune response to *Chlamydia trachomatis*. The antibody rise occurs too late to be useful in managing acute infections but provides evidence of retrospective infection. Additionally, many commercially available serological diagnostic kits are expensive and often lack both sensitivity and specificity, limiting their effectiveness.[17]

Methods based on the detection of the bacterial nucleic acid like ligase chain reaction (LCR) and polymerase chain reaction (PCR) are the most sensitive diagnostic methods available for detecting chlamydia infection and can be used with non-invasively collected specimen. These methods are the first non-culture assay to surpass culture sensitivity.[18]

The aim of this study was to assess the prevalence of *C. trachomatis* among pregnant women who attend ante-natal clinics in Kaduna Metropolis using molecular techniques. Moreover, to evaluate *C. trachomatis* infection rate associated with socio-economic status of the participants enrolled in the study.

Method

Study Area

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The research was conducted at three locations within the Kaduna metropolitan area: Yusuf Dantsoho Memorial Hospital, General Hospital Kawo and the Kaduna Polytechnic Medical Centre. The hospitals cater for lower- and middle-class population within the study areas.

Study population and clinical specimen

A cross-sectional study design was implemented in this study. All pregnant women who attended the ante natal clinics within the period of study were enrolled for the study. Samples were collected from a total of 200 pregnant women receiving care at these clinics. Individual consent was obtained and patient confidentiality was strictly maintained. Ethical approval was obtained from the hospital. Information such as age, occupation, educational qualification and source of income was obtained from the participants by interview method. Pregnant women who used antibiotics in the preceding month were excluded.

Laboratory procedures

Five millilitre (5 ml) of blood samples were obtained from the participants by venipuncture using sterile disposable needles and syringes into well labelled tubes. The blood samples were stored in a cold box and transported to the laboratory for analysis. The blood samples were left to clot and centrifuged at 3,000 rpm. Thereafter, the sera were separated into bottles and preserved at 4^{O}C. Antibody against *C. trachomatis* was determined using *C. trachomatis* IgG ELISA Trinity kits, (Cat No 2346200, USA). Microtiter strips wells were precoated with *Chlamydia trachomatis* antigen to bind corresponding antibodies of the specimen. To eliminate any unbound sample material, the wells were thoroughly washed. Subsequently, a conjugate consisting of horseradish peroxidase (HRP)-labeled anti-human IgG was introduced. This conjugate specifically bound to the Chlamydia-specific antibodies already captured in the wells. To visualize the immune complex formed by this binding, Tetramethylbenzidine (TMB) substrate was added, resulting in the development of a blue-colored reaction product. Finally, the reaction was halted by adding sulfuric acid. Absorbance was read at 450nm using an ELISA micro well plate reader.

DNA Extraction

To a 1.5ml microcentrifuge tube, 200µl of blood was added, then 400µl of lysis buffer and 10µl proteinase K, the tube was placed on a heat block at 60^{0}C for 1hour. Thereafter, 400µl of phenol chloroform (1:1) was added to the lysate and vortexed briefly. This was centrifuged at 13000rpm for 10minutes in an Eppendorf 5415D microfuge to separate the phases. The upper layer was carefully removed with a micropipette and added to a new 1.5ml microcentrifuge tube. Then 400µl of chloroform was added to the recovered clear layer and was vortexed briefly. This was centrifuged at 13000rpm for 5minutes to separate the phases. The upper layer was carefully removed with a pipette and added to a new 1.5ml tube. Equal volumes of 100% ethanol and 20µl of 3M sodium acetate was added and mixed by inverting the tube several times. This was incubated at -20^{o}C overnight. The tube was then spun at 13,000rpm for 10 minutes in refrigerated centrifuge. The ethanol was removed and 400µl of 70% ethanol was added. This was centrifuged at 13000rpm for 5 minutes at 4^{o}C. This step was repeated to ensure that all the salt was removed. The DNA was dried by leaving the tube open for 10 minutes and the DNA pellet was re-suspended in 50µl molecular grade water.

Primers used in the study

One pair of oligonucleotide primers specific for a 144 region of the *Chlamydia trachomatis* gene coding for the major outer membrane protein (MOMP) was selected. The sequences from 5′ to 3′ of these oligonucleotide primers are as shown in Table 1.

Table 1: Primers used in the Study

Primer	Strand	Sequence(5′-3′)	Position
MOMP87	Sense	TGAACCAAGCCTTATGATCGACGGA	87 - 111
C214	Antisense	TCTTCGAYTTTACCTTTAGATTGA	648 - 671

PCR

In vitro amplification of DNA was performed in 0.5ml thin-walled microfuge tubes containing the PCR premix (Bioneer, South Korea) by adding 1µl each of forward and reverse primers, 1µl of DNA template and17µl of molecular grade water. Samples were then placed in a Biometra® T3 thermocycler and subjected to the following reaction conditions: Pre-Denaturation at 95^{0}C

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for 5min, Denaturation at 94^{0}C for 1 min, Annealing at 52^{0}C for 1 min, Extension at 72^{0}C for 1 min, 25 cycles and Final extension at 72^{0}C for 7min. A positive control (DNA of *C. trachomatis*) and a negative reagent control (distilled water) were included in the PCR run.

Agarose gel Electrophoresis

DNA was separated according to its size via electrophoresis on 1.5% (w/v) agarose in 1 x Tris acetate EDTA (TAE) buffer (40mM Tris base, 20mM acetic acid, 1mM EDTA pH8.0) containing 5µl of ethidium bromide (NBS Biologicals, UK). DNA samples were mixed with 5µl of DNA loading buffer (NEB, USA) and loaded onto the gel, DNA Molecular weight marker was also loaded to the first lane of the gel. Electrophoresis was carried out in a BioRad electrophoresis chamber at 100V for 60 min. DNA was visualized with a UV transilluminator.

Statistical Analysis

Data obtained were statistically described in terms of mean ± standard deviation and percentages. Chi-square and Fishers exact two-tailed test were used to test for the association between *Chlamydia trachomatis* infection and socio-economic status such as age group of subjects, education level, income and marriage type. $P < 0.05$ was considered significant throughout the study.

Results

Data showed that of the 200 sera samples obtained from pregnant women attending antenatal clinics and tested for *C. trachomatis* antibody by ELISA, 19 were positive while 181 returned negative giving a prevalence rate of 9.5%. However, of the 10 sera samples that returned positive for *C. trachomatis* by ELISA and were tested by PCR, only 4 were positive for *Chlamydia trachomatis omp1* gene giving a prevalence of 3.8% as shown in Table 2.

Table 2: Detection of *C. trachomatis* in the sera samples of participants using ELISA and PCR

Method	Sample Size	Positive	Negative	%
ELISA	200	19	181	9.5
PCR	10	4	6	3.8

Agarose gel electrophoresis showed that *omp1* gene was amplified in 4 of the 10 sera samples that were positive for *C. trachomatis* using serology, as shown in Figure 1.

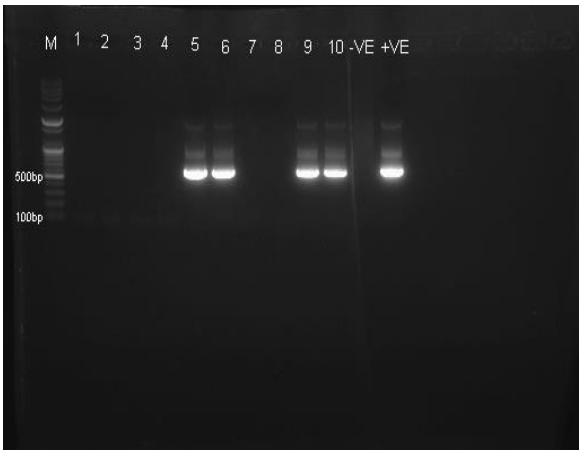

Figure 1: Agarose gel electrophoresis of the amplified *omp1* gene of *C. trachomatis*
Key:
M=Molecular marker
-VE=Negative control
+VE= Positive control

Correlation of the sociodemographic factors and chlamydia infection detected by molecular approach showed that there was significant association between chlamydia infection and factors such as age, level of income, educational qualification and marriage type, with probability values for each less than 0.05 using chi square and fishers test for PCR respectively as shown in Table 3.

Table 3: Association of socio demographic factors among pregnant women with Chlamydia infection using PCR as diagnostic tool

	PCR Positive %	PCR Negative%	Fisher's test	P-value
Women's age				
≤ 25	3(50)	1(25)		
26 – 35	2(34)	2(50)	9.342	0.009
36 – 45	1(16)	1(25)		

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Income /month				
< 20,000 (low)	4(67)	1(25)		
20,000 – 50,000	1(16)	1(25)	7.342	0.005
> 50,000 (high)	1(16)	2(50)		
Educational Qualification				
Illiterates/primary	3(50)	2(50)		
Secondary	1(16)	1(25)	4.342	0.006
Tertiary	2(34)	1(25)		
Marriage type				
Monogamous	2(34)	3(75)		
Polygamous	4(66)	1(25)	8.442	0.010

Discussion

Genitourinary tract infections caused by *C. trachomatis* are a major cause of morbidity in sexually active individuals especially in women.[19] The organism is responsible for variety of infections in women like cervicitis, endometritis, acute urethral syndrome and salpingitis[20] and infection during pregnancy may lead to serious complications. Research indicates that the prevalence and incidence of *Chlamydia trachomatis* (CT) infections tend to decline when screening programs are implemented. The Centers for Disease Control and Prevention (CDC) has endorsed antenatal screening as an effective measure to detect and manage CT infections during pregnancy.[21].Antenatal screening, as recommended by the CDC would be beneficial to decrease morbidity amongst women, but also to prevent vertical (infant) and horizontal (partner) transmission.

Data obtained from this work showed that only 4 of the 10 sera samples that tested positive for *C. trachomatis* antibody by ELISA, returned positive with PCR technique. Although ELISA technique is widely used for the detection of *C. trachomatis*, it may lack sensitivity and specificity in comparison with PCR techniques thus, the prevalence rate using the serological approach is probably over estimated. The true sensitivity of non-molecular diagnostic testing for *C. trachomatis* is predicated on the quality of the reference standard and could demonstrate falsely elevated performance characteristics.[22] Molecular genetic methods on the other hand are rapid and reliable screening measures for *C. trachomatis*. This is particularly effective in regions where the disease prevalence is notably high.

To identify some of the predisposing factors associated with chlamydia infection, several determinants were studied. It was found that age, economic status, educational qualification and marriage type were substantially linked to a chlamydia infection. The significant association of income and education with positive Chlamydia infection is consistent with the findings of Behrozi et al.[23] This could be attributed to socio economic status of the subjects, who for lack of awareness or may be visiting those clinics for the first time to receive treatment for health issues because of poverty.

Similarly, the strong correlation between chlamydia infection and young individuals (less than 25 years old) concurred with the study of Sethi et al.[13] According to O'Connell and Ferone,[24] rates of reported cases of chlamydia are highest among adolescents and young adults aged 15–24 years. The impact of age may be linked to sexual factors, including the frequency of sexual activity with a partner, the number of partners, and the duration of each encounter.[13]

The reduced susceptibility to infections observed with increasing age has been linked to changes in epithelial tissues, potentially leading to a lower infection rate among older individuals.[25]

Implications of the findings of this study

Nucleic acid testing (NAT) for C. trachomatis enables highly specific C. trachomatis detection with sensitivity levels significantly improved compared with the serological assays for the antibody. This technique, in addition confirms active infection. Implementing NAT testing in antenatal clinics has the potential to significantly improve control of chlamydial infections in pregnancy and reduce the complications associated with such infections.

Strengths and Limitations of the Study

Given that Nucleic acid amplification tests are expensive, only 10 samples were subjected to NAT.

Conclusion

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In this study, genital Chlamydia trachomatis infections were found to be more prevalent among women under 25 years of age. Additionally, a significant correlation was identified between the occurrence of chlamydia infections and factors such as income level and educational attainment. Furthermore, the specificity of PCR over ELISA was demonstrated as only 4 out of the 10 samples that tested positive with ELISA gave positive results with PCR. As nucleic acid-based diagnostic tests continue to advance, there is need to expand their availability and implementation in clinical laboratories of all scales, both small and large, particularly in developing nations such as Nigeria.

Declarations

Ethical Consideration: Ethical approval was obtained from the Kaduna State Ministry of Health.

Authors' Contribution: ENE and BBF conceived and designed the research, BBF collected the data, ENE, BBF, AMI and MGL analysed the data. ENE and BBF wrote the manuscript, all authors reviewed the manuscript.

Conflict of interest: The authors declare no conflict of interest.

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