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Epidemiological Assessment of Chlamydiasis among Women in Jalingo and Kurmi Local Government Areas, Taraba State, Nigeria

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Abstract

Bacterial sexually transmitted infections remain a major public health concern among adults globally. This study investigates the prevalence and associated risk factors of *Chlamydia trachomatis* infection among women in Jalingo and Kurmi Local Government Areas of Taraba State, Nigeria. Employing a cross-sectional design, 370 women aged 18–55 years were recruited from selected hospitals and clinics. Data collection involved structured questionnaires and laboratory analysis of endocervical swab samples using direct immunofluorescent assay (IFA) and polymerase chain reaction (PCR) for confirmation. The overall prevalence of *C. trachomatis* infection was 21.8%, with a higher rate in Jalingo (25.5%) compared to Kurmi (18.0%). The highest prevalence was recorded among women aged 18–24 years and those reporting multiple sexual partners. Other statistically significant risk factors included low educational attainment, inconsistent condom use, and a prior history of sexually transmitted infections. The findings underscore the necessity of integrating sensitive diagnostic methods, such as PCR, into routine STI screening to improve early detection and treatment outcomes. The study

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further emphasizes the need for targeted public health interventions, including routine chlamydia screening, comprehensive sexual health education, and improved access to sexual and reproductive health services in the region.

Keywords: Epidemiology; *Chlamydia trachomatis*; STIs; Women's Health; PCR; IFA; Taraba

INTRODUCTION

Chlamydiasis is a sexually transmitted bacterial infection that poses a significant public health challenge globally (Elendu *et al*., 2024). If left untreated, it can lead to serious complications and irreversible damage to the reproductive system, including infertility (Tsevat *et al*., 2017). The infection is responsible for various urogenital conditions such as cervicitis, pelvic inflammatory disease (PID), urethritis, epididymitis, prostatitis, and lymphogranuloma venereum (Grygiel-Górniak & Folga, 2023). In sub-Saharan Africa, including Nigeria, previous studies have reported a high prevalence of *Chlamydia trachomatis* infections, particularly among sexually active individuals under the age of twenty five (25) (Sohaili *et al*., 2024). The infection disproportionately affects women, often with asymptomatic presentations, leading to severe consequences for reproductive health, including infertility and ectopic pregnancies (Van Gerwen *et al*., 2022). Socio-economic disadvantages have also been observed among men and women affected by the infection, with a profound impact on their quality of life and economic productivity (Dukers-Muijrers *et al*., 2022).

Despite the significant burden of chlamydiasis, there is a lack of awareness and understanding of the disease among young adults (Voyiatzaki *et al*., 2021). Studies, such as those by (Workowski *et al*., 2021), have highlighted a low level of knowledge regarding the symptoms, transmission routes, and importance of early diagnosis and treatment, further complicating efforts to control the spread of chlamydia disease. Globally*, C. trachomatis* is estimated to be the primary cause of approximately 90 million cases of sexually transmitted diseases annually (Fu *et al*., 2022). Moreover, women with a history of *Chlamydia trachomatis* infection, particularly serotype G, are reported to have a 6.5-fold increased risk of developing cervical cancer (Bhuvanendran Pillai *et al*., 2022). Given the burden of chlamydiasis and its associated complications, this study was conducted to assess the prevalence of *C. trachomatis* infection among women attending gynecology and family

planning clinics in Jalingo and Kurmi Local Government Areas of Taraba State, Nigeria. The study also aimed to examine socio-economic and socio-demographic factors influencing infection rates and to evaluate the diagnostic accuracy of laboratory methods, including the direct immuno-fluorescent Analysis and polymerase chain reaction (PCR).

MATERIALS AND METHODS

Study Area

The study areas were conducted in Jalingo and Kurmi Local Government Areas (LGAs), Taraba State, Nigeria. Jalingo LGA (8.9°N; 11.3°E) is the capital city located at the northern zone of Taraba State with a savannah vegetation, while Kurmi LGA (8.5°N and 11.5°E) is located at central zone of the State with a forest vegetation. Jalingo LGA has a dry season from mid-October to May mid-April, while the rainy season is from May to October. Kurmi LGA has a dry season from October to March, and the rainy season from April to October among sexually active women and the diagnosis of the specimen was carried out at Taraba Specialist Hospital Jalingo, Taraba State, Nigeria.

Ethical clearance

Ethical Approval for the study was obtained from the Ethical Review Committee on Human Research (ERCHR), Ministry of Health Jalingo, Taraba State, Nigeria with Reference Number: RE/FMC/00/00.

Sampling method and determination of sample size

Study participants were randomly selected from sexually active women attending Taraba Specialist Hospital, Jalingo, and General Hospital, Kurmi LGA, Taraba State, Nigeria. The sample size was determined using the formula:

$$n = \frac{z^2 * p * (1-p)}{e^2}$$

Where;

- Z is the statistic corresponding to level of confidence (1.96)
- p is the previous prevalence (70.3%) of *C. trachomatis* infection in Northeast Nigeria (Arije *et al.*, 2024).
- e^2 is the desire level of precision (that is the margin of error) (0.05)

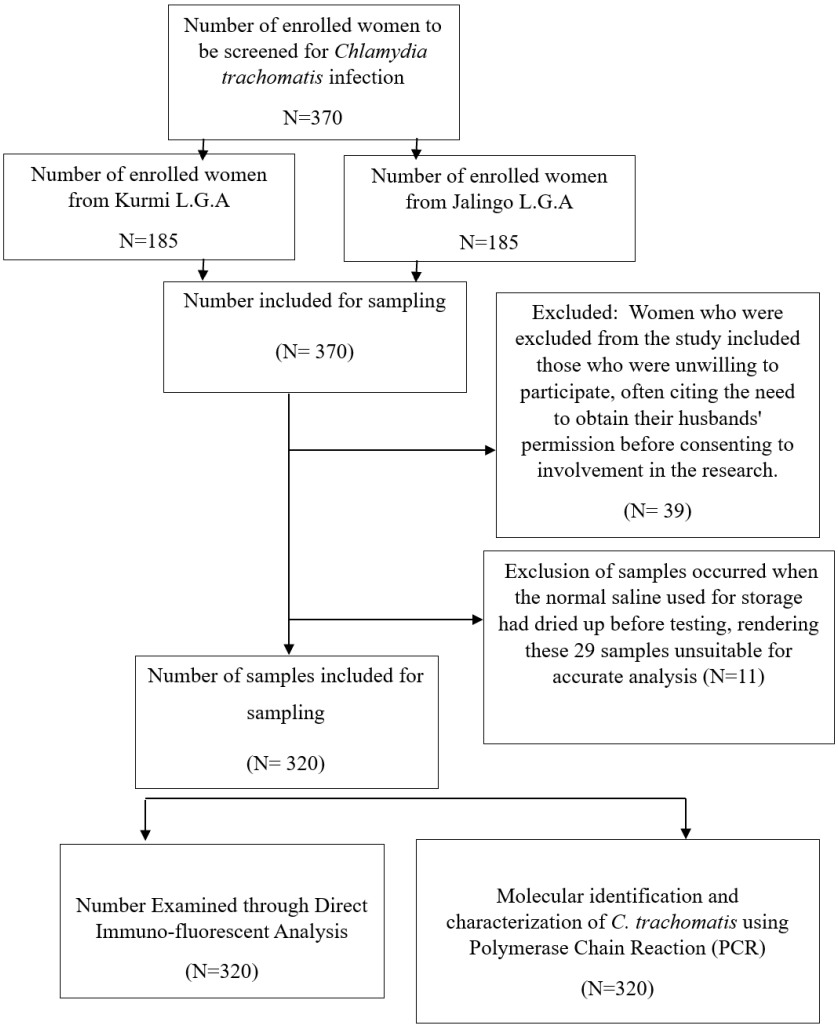
- q = 1-p

Sample size (n) =1.96^2*0.703*(1-0.703)/ (0.05^2)

= **320.83**

Inclusion and exclusion criteria

The inclusion criteria for the study were self-reported healthy women aged 20 to 55 years who were sexually active, willing to provide specimens, and had not used azithromycin, erythromycin, doxycycline, or tetracycline in the past three months. Exclusion criteria included women who refused consent, had undergone hysterectomy or cervical conisation, were menstruating or had vaginal bleeding at the time of specimen collection, had been treated for premalignant cervical lesions, or had received antineoplastic or immunotherapy. Additionally, participants needed a prior history of penetrative vaginal intercourse.



Flow chart: Exclusion and inclusion of women for *Chlamydia trachomatis* infection

Data Collection

The public health importance of the study was explained to all selected participants in English language and local language, highlighting the awareness as relate to cervical cancer, its warning symptoms, its complications and prevention in details. A midwife /nurse explained the procedure involved in the sample collection to study participants. A structured questionnaire and laboratory testing were used to collect demographic data and detect *C. trachomatis* infection.

Questionnaire administration

The questionnaires were administered to participants who consented to the research during sample collection. The questionnaire, designed to evaluate socio-demographic, socio-economic, and knowledge-related factors, was structured into five sections. Section A captured participants' biodata, including their local government area, age group, religion, education level, marital status, pregnancy plans, age at first pregnancy, and history of sexually transmitted infections (STIs) (Azim *et al*., 2023). Section B assessed participants' knowledge about *Chlamydia trachomatis* infection, covering awareness of asymptomatic infections, cure possibilities, reinfection potential, effects on fertility, preventive measures like condom use, and the presence of symptoms (Bandhu Kalanidhi *et al*., 2021). Section C examined perceptions of transmission routes, such as genital, anal, and oral contact with infected individuals, as well as non-sexual routes like sharing towels or using the same toilet seat (Toledano-Toledano *et al*., 2019). Section D explored attitudes toward Chlamydia testing, focusing on the necessity and scope of testing for pregnant women, including opinions on testing those at increased risk or only those desiring testing (Ocansey *et al*., 2021). Lastly, Section E evaluated participants' emotional responses to being offered a test, including feelings of satisfaction, surprise, stigma, shame, or neutrality. These detailed responses provided critical insights into the socio-demographic context, knowledge levels, attitudes, and emotional experiences related to *Chlamydia trachomatis* among women in Jalingo and Kurmi Local Government Areas of Taraba State, Nigeria

Collection and processing of pap smear and cervical exfoliated cells

In the laboratory, Pap smear collection was performed using a wooden spatula to obtain cervical fluid from women, which was then rinsed by squashing in sterile normal saline contained in universal containers and stored before processing. Additionally, two swabs were collected from each participant; one for *Chlamydia trachomatis* screening by PCR

and the other for *C. trachomatis* culture (Cao *et al*., 2022). The cervical exfoliated cells were collected using a cytobrush, with scraping from the cervix and endocervical region transferred into a tube containing Roswell Park Memorial Institute (RPMI) medium (Chong *et al*., 2020). These samples were incubated at 4°C for 24 hours before being washed with phosphate-buffered saline (PBS) (Zhang *et al*., 2024). Cervical samples were subsequently stored in RNA at -80°C for future analysis. For genomic analysis, the genomic DNA (gDNA) was extracted using the Genomic DNA Mini Kit (Blood/Bacteria/Cultured Cells) (Wu *et al*., 2019). The swab designated for *C. trachomatis* culture was inoculated into 1 mL of chlamydia transport medium, placed on ice, and transported to the laboratory for same-day processing (Zhou *et al*., 2024). The swab intended for PCR screening was stored in PBS and refrigerated at 2–8°C until processing (Zhou *et al*., 2024). This systematic approach ensured the integrity and reliability of the collected samples for further diagnostic and molecular analysis

Processing of cervical exofliated cells

The cervical exfoliated cells were collected with cytobrush by scraping using Cervix-Brush and the endocervical curettage into a tube containing Roswell Park Memorial Institute (RPMI) medium incubated at 4°C for 24hours (Simonsen *et al*., 2016). After washing with phosphate-buffered saline (PBS), cervical samples were stored in Ribonucleic Acid (RNA) at -80°C. Once thawed, the genomic Deoxyribonucleic Acid (gDNA) was extracted using Genomic DNA Mini Kit (Blood/ Bacteria/Cultured Cells). One for *Chlamydia trachomatis* screening by PCR, and the other for *C. trachomatis* culture. Cervical exfoliated cell smears were immediately fixed in 95% ethanol and transferred to the laboratory, where they were processed using the George Papanikolaou staining protocol (Goel *et al*., 2020); additionally, smears prepared from cervical specimens collected with an Ayre's spatula were wet-fixed, stained, and examined microscopically, while fluorescent antibody staining of a second smear using species-specific antibodies enabled detection of *Chlamydia trachomatis* inclusion bodies under a fluorescent microscope (Enechukwu *et al*., 2021). The swab was used for culture was inoculated into 1 ml chlamydia transport medium, placed on ice and transported to the laboratory for processing on the same day. The swab that was used for screening was placed in phosphate buffer saline (PBS) and kept in the fridge at 2-8°C until processing. Cervical swabs were cultured on McCoy cell monolayers in shell vials, incubated with supplemented DMEM, and processed to produce stock inoculant stored at -80°C; inclusion-forming units (IFU) were visualized using the

MicroTrak *Chlamydia trachomatis* culture confirmation test. Minimal inhibitory concentrations (MICs) of azithromycin and doxycycline were determined by overlaying infected monolayers with serial antimicrobial dilutions, incubating for 48 hours, and quantifying inclusions microscopically to establish the lowest concentration with no visible inclusions

Direct immunofluorescence assay (DIFA)

In this method, the exfoliated swab was preserved in micro tubes containing PBS at 2 to 8°C, it was centrifuged for 15 min at 3000rpm with a micro-centrifuge apparatus. The supernatant was discarnate, and smear made on grease-free microscopic slide. It was air-dried for 5 to 10 min and fixed with 70% methanol (0.5 ml) for 15min. A drop conjugated antibody onto the test and control slide, covering the specimen surface thoroughly in a wet jar at room temperature in dark area for 15 min (Bayerl *et al.*, 2023). The slides were washed in a beaker with distilled water for 15 to 20s, and allowed to air-dry (Budge *et al.*, 2024). A drop of the mounting media was placed on the test and control slide and covered with coverslip. The slides were examined using immunofluorescence microscope (Schmid *et al.*, 2021). The slides can be preserved in a dark place at 2 to 8°C for a maximum of 24 h (Sato *et al.*, 2021). First, positive and negative control slides were observed, while the test slides were observed afterwards. DFA monoclonal antibody that was performed from the stained Pap smear of positive *C. trachomatis* was observed.

Polymerase Chain Reaction (PCR) and Gel Electrophoresis for Molecular Identification of *Chlamydia trachomatis*

Polymerase Chain Reaction (PCR) Procedure

The molecular identification and characterization of *Chlamydia trachomatis* from urine samples were performed using Polymerase Chain Reaction (PCR) (Sethi *et al.*, 2017). DNA was extracted from the samples using a commercial DNA extraction kit following the manufacturer's protocol. The extracted DNA was then subjected to PCR amplification to target specific gene regions associated *with C. trachomatis* (Korhonen *et al.*, 2019).

- The PCR reaction mixture was prepared in a final volume of 50 μL, consisting of:
- 25 μL of 2X PCR master mix (including Taq DNA polymerase, dNTPs, and $MgCl_2$),
- 2 μL of each forward and reverse primer (10 pmol each),
- 5 μL of template DNA,

- 16 μL of nuclease-free water.

The amplification was carried out in a thermal cycler under the following conditions:

Initial denaturation at 95°C for 5 minutes at 35 cycles of:

- Denaturation at 94°C for 30 seconds,
- Annealing at 55°C for 45 seconds,
- Extension at 72°C for 1 minute.
- A final extension at 72°C for 10 minutes (Jiang *et al.*, 2021).

The amplified DNA fragments were stored at -20°C until further analysis.

Gel Electrophoresis for DNA Fragment Elucidation

To confirm the amplification of DNA fragments, agarose gel electrophoresis was performed (Bogiel *et al.*, 2022). A 2% agarose gel was prepared using TAE (Tris-Acetate-EDTA) buffer, and electrophoresis was conducted at 90V for 30 minutes. The gel was stained with Ethidium Bromide to visualize the DNA under a UV trans-illuminator. A 100-kb DNA ladder (Promega) was used as a molecular weight marker to estimate the size of the PCR products (Cunha *et al.*, 2020). DNA bands were analyzed and compared against the marker to confirm the expected product sizes.

Restriction Endonuclease Digestion and Fragment Analysis

The *Chlamydia trachomatis* genome consists of 1,042,519 nucleotide base pairs, with approximately 894 protein-coding sequences (Luu *et al.*, 2023). The genome was digested using restriction endonucleases with 8-bp recognition sequences to generate relatively few fragments. Double digestion with selected enzymes revealed six fragments, two of which contained co-migrating fragments. The fragment sizes ranged from 555bp to 10 kb. To achieve optimal separation, pulsed-field gel electrophoresis (PFGE) was performed with a pulse time ramp of 15s to 75s over 20 hours. The restriction endonuclease Sse8387I (CCTGCAIGG) cleaved the *C. trachomatis* genome into 17 fragments, with sizes ranging from 220bp to 9kb. Of these, 10 bands ranged from 65 kb to 21kb. Separation of these bands required a pulse time ramp of 1s to 15s over 20 hours. The sum of the fragment sizes for each enzyme differed by less than 2%, and the calculated chromosome size was 1,045 kb, confirming genome integrity and restriction digestion efficiency. The lane distribution in gel electrophoresis was as follows:

- Lane 1: 100-kb DNA ladder (Promega),

- Lane 2: Undigested *C. trachomatis* genomic DNA,
- Lane 3: PCR-amplified DNA fragments,
- Lane 4: DNA digested with Sse8387I,
- Lane 5: DNA digested with an additional restriction enzyme,
- Lane 6: Double digestion with both enzymes.

The visualization of distinct bands in the gel electrophoresis confirmed the successful amplification and digestion of *C. trachomatis* DNA, aiding in molecular characterization.

Statistical analysis

All data were analyzed using SPSS version 22. Descriptive statistics was used to analyze the demographic variables of all participants and their screening results including *C. trachomatis* distribution and results were presented in frequency and tables. Chi square test was used to test for association differences variables. Statistical test of association was carried out with *P* values ≤ 0.05 considered significant.

RESULTS

Number of participants in the study

Table 1 presents the number of participants in the study indicating that out of 370 women participants from Jalingo and Kurmi Local Government Areas in Taraba State, Nigeria, 320 (86%) were included in the study, while 50 (14%) were excluded. The primary reasons for exclusion were unwillingness to participate, often due to the need for their husbands' permission (39 women, 11%), and loss of samples due to drying up of normal saline during storage before testing (11 women, 3%).

Table 1: The number of participants in the study

Variable	Number of Participants (N)	Percentage (%)
Total Participants	370	100%
Included in Study	320	86%
Excluded from Study	50	14%
Reason for Exclusion		
Unwillingness to participate, often citing the need to obtain their husbands' permission before consenting to involvement in the research	39	11%
Lost to drying up of normal saline in sample during storage before testing	11	3%

Demographic Characteristics and Awareness of *Chlamydia trachomatis* Infection

Table 2 depicts the result on the demographic characteristics and awareness of *Chlamydia trachomatis* infection among participants. The data shows that the prevalence of *Chlamydia trachomatis* infection among the examined women was relatively low and did not significantly differ across various demographic and behavioral factors, as indicated by the high p-values (>0.05). Specifically, infection rates were similar between women from Jalingo (9.9%) and Kurmi (6.9%), with no statistically significant difference (p=0.331). Age, religion, education level, marital status, pregnancy planning methods, age at first pregnancy, and STI history (syphilis or gonorrhea) showed no significant association with infection status, suggesting that these factors do not strongly influence Chlamydia prevalence in this population within the study's context.

Table 2: Demographic Characteristics and Awareness of *Chlamydia trachomatis* Infection

Variables	*Chlamydia trachomatis* infection (%)			
	No Examined	No. Positive	x^2	P. Value
LGA				
• Jalingo	160	16(9.9)	0.944	0.331
• Kurmi	160	11(6.9)		
Age (years)				
• 15-24	30	2(6.7)	3.556	0.314
• 25-34	91	8(8.8)		
• 35-44	151	16(10.6)		
• 45-54	48	1(2.1)		
Religion				
• Islam	118	13(11.0)	1.611	0.447
• Christian	188	13(6.9)		
• Others	14	1(7.1)		
Education Level				
• No Formal Education	71	7(9.9)	0.442	0.931
• Primary Education	60	4(6.7)		
• Secondary Education	92	8(8.7)		
• Tertiary Education	97	8(8.2)		
Marital Status				
• Married	182	17(9.3)	0.873	0.832
• Single	67	4(6.0)		
• Divorced	42	4(9.5)		

•Widow	29	2(6.9)		
Pregnancy plan				
• Contraceptive	52	7(13.5)	3.921	0.417
• Condom	93	6(7.1)		
• Withdrawal	135	8(5.9)		
• Injection	40	5(12.5)		
• VTU	9	1(11.1)		
Age of first Pregnancy (years)				
• 15-24	71	6(8.5)	3.138	0.535
• 25-34	113	10(8.8)		
• 35-44	64	8(12.5)		
• 45-54	42	2(4.8)		
• 55-64	30	1(3.3)		
STI History				
• Syphilis	104	9(8.7)	2.117	0.833
• Gonorrhea	24	3(2.8)		

Source: Field study

Knowledge Concerning *Chlamydia trachomatis* Infection Using Antimicrobial Assay

Table 3 presents the association between women's knowledge of *Chlamydia trachomatis* and their infection status. A total of 320 women were examined across various knowledge variables. 8.9% of those who knew that chlamydia can be transmitted without symptoms tested positive, compared to 7.8% of those who did not know. The difference was not statistically significant (p = 0.717). Among women who knew chlamydia can be cured with medicines, 7.1% were positive, compared to 8.9% among those who did not. This difference was also not significant (p = 0.619). 6.8% of those aware of the possibility of reinfection tested positive versus 9.8% of those unaware (p = 0.332). No significant association was found. Knowledge of infertility as a consequence showed a possible data inconsistency, with a surprisingly high 87.3% positivity among those who answered "No." 6.3% positivity was recorded among those who believed condoms are protective versus 9.0% among those who did not. However, this difference was not statistically significant (p = 0.481). Equal positivity rates (6.5%) were observed among those who believed chlamydia always shows symptoms and those who did not. This suggests that symptom-based assumptions do not correlate with actual infection (p = 0.531). Overall, none of the knowledge indicators showed a statistically significant association with *C. trachomatis*

infection (all p-values > 0.05). This indicates that knowledge alone does not significantly reduce infection risk, pointing to the importance of behavioral change, access to screening/treatment, and public health interventions beyond awareness campaigns.

Table 3: Knowledge of *Chlamydia trachomatis* Infection among women examined in Jalingo and Kurmi LGAs, Taraba State, Nigeria

Variables		*Chlamydia trachomatis* infection (%)		
	No Examined	No. Positive	% 95 CI	P. Value
Can you infect people without knowing it?				
• Yes	191	17(8.9)	0.503-2.612	0.717
• No	129	10(7.8)		
Can chlamydia be cured with medicines?				
• Yes	84	6(7.1)	0.307-2.131	0.619
• No	236	21(8.9)		
Can you have chlamydia more than once?				
• Yes	147	10(6.8)	0.331-1.746	0.332
• No	173	17(9.8)		
Can chlamydia cause infertility?				
• Yes	73	22(8.9)		0.578
• No	247	144(87.3)		
Is the use of condom protective against chlamydia?				
• Yes	64	4(6.3)		0.481
• No	256	23(9.0)		
Will you always have symptoms when infected?				
• Yes	62	4(6.5)		0.531
• No	258	23(6.5)		

Source: field study

Transmission of *Chlamydia trachomatis* Using Antimicrobial Assay

Table 4 presents result on the association between self-reported exposure to various transmission routes and the prevalence of *Chlamydia trachomatis* infection among women in Jalingo and Kurmi Local Government Areas of Taraba State. The analysis

indicates that among various transmission methods for *Chlamydia trachomatis*, only anal sexual contact shows a statistically significant association with infection (p=0.029), with a lower infection rate (4.8%) among those engaging in anal sex compared to 11.6% among those who did not, and an adjusted odds ratio (aOR) of 0.327 suggesting a reduced risk. Other transmission routes, such as genital sexual contact, oral sex, kissing, sharing toilet seats, and sharing bath towels, did not show significant associations with infection (all p-values >0.05). This suggests that, within this population, anal sexual contact may have a different or more complex role in transmission dynamics, but overall, no strong evidence points to these other common behaviors as significant risk factors for Chlamydia infection in this study.

Table 4: Ways of Transmission of *Chlamydia trachomatis* infection in relation to various transmission ways among examined women in Jalingo and Kurmi LGAs, Taraba State, Nigeria.

Variables		*Chlamydia trachomatis* infection (%)			
	No Examined	No. Positive	aOR	% 95 CI	P. Value
Genital sexual contact with an infected person					
• Yes	132	11(8.3)	1.284	0.545-3.024	0.955
• No	188	16(8.5)			
Anal sexual contact with an infected person					
• Yes	147	7(4.8)	0.327	0.148-0.949	0.029
• No	173	20(11.6)			
Oral sexual contact with an infected person					
• Yes	191	17(8.9)			0.717
• No	129	10(7.8)			
Kissing an infected person on the mouth					
• Yes	116	9(7.8)	0.886	0.381-2.060	0.742
• No	204	18(8.8)			
Using same toilet seat					
• Yes	84	6(7.1)			0.619
• No	236	21(8.9)			
Sharing bath towels with an infected person					
• Yes	73	5(6.8)			0.578
• No	247	22(8.9)			

Source: field study

The Attitudes and Experiences of Women for Chlamydial Testing in Taraba State

Table 5 presents the relationship between women's attitudes and emotional responses toward *Chlamydia trachomatis* testing and their corresponding infection status. The goal is to assess whether perceptions and experiences with testing influence or correlate with actual infection rates. Women who believed that all pregnant women should be tested had a slightly lower infection rate (6.8%) compared to those who disagreed (9.8%), but this difference was not statistically significant (p = 0.941). Similar trends were observed for those who supported testing only for: women at increased risk (p = 0.309), women who voluntarily request it (p = 0.309), and those opposing testing during pregnancy (p = 0.443) respectively. However, across all attitude-related variables, there were no significant associations between beliefs about testing criteria and infection status.

Women who expressed satisfaction with the test offer had a slightly lower infection rate (6.5%) than those who did not (8.9%), but the difference was not statistically significant (p = 0.393). Women who felt surprised by the offer had similar infection rates (8.3%) compared to those who did not (8.5%); again, not statistically significant (p = 0.003*). Those who reported feeling: stigmatized (6.8% vs. 9.0%; p = 0.516), ashamed (7.8% vs. 8.8%; p = 0.109), Or had no emotional impact (8.9% vs. 7.8%; p = 0.131) but all differences were not statistically significant. This implies emotional reactions whether negative or neutral did not significantly influence infection rates, suggesting such attitudes may not affect risk directly, though they may impact acceptance of screening programs.

Table 5: Attitudes and Experiences of Women Regarding *Chlamydia trachomatis* Test in Taraba State

Variables	*Chlamydia trachomatis* infection (%)		
	No Examined	No. Positive	P. Value
All pregnant women should be tested			
• Yes	147	10(6.8)	0.941
• No	173	17(9.8)	
Only pregnant women at increased risk			
• Yes	73	5(6.8)	0.309
• No	247	22(8.9)	
Only pregnant woman who want to be tested			
• Yes	71	5(6.8)	0.309
• No	249	22(8.9)	
Testing pregnant women is not necessary			
• Yes	63	4(6.3)	0.443
• No	257	23(8.9)	
No opinion			
• Yes	50	3(6.0)	0.456

• No	270	24(8.9)	
I felt satisfied with the test offer			
• Yes	62	4(6.5)	0.393
• No	258	23(8.9)	
I felt surprised by the test offer			
• Yes	132	11(8.3)	0.003
• No	188	16(8.5)	
I felt stigmatized by the test offer			
• Yes	73	5(6.8)	0.516
• No	247	22(9.0)	
I felt ashamed by the test offer			
• Yes	116	9(7.8)	0.109
• No	204	18(8.8)	
The test offer had no emotional impact on me			
• Yes	191	17(8.9)	0.131
• No	129	10(7.8)	

Source: field study

Assessment of immuno-fluorescent Analysis on *Chlamydia trachomatis* Infection on women in Taraba State

Table 6 shows findings from immuno-fluorescent analysis used to detect *Chlamydia trachomatis* infection among women in the two Local Government Areas (LGAs): Jalingo and Kurmi, each with 160 women examined. In Jalingo LGA; 25 out of 160 women tested positive, representing a prevalence of 15.8% and 135 women (84.2%) tested negative while in Kurmi LGA, 16 out of 160 women tested positive, yielding a prevalence of 9.8% and 144 women (90.2%) tested negative. An overall prevalence of 21.8% was recorded, with Jalingo (25.5%) showing the highest rate compared to 18.0% in Kurmi and a Chi-square value (χ^2) of 1.012 indicating that this difference is not statistically significant. The result suggests that, although there is a numerical difference in infection rates between the two LGAs, this variation could be due to random chance rather than a true underlying difference in infection burden.

Table 6: Immuno-fluorescent Analysis on *Chlamydia trachomatis* Infection on women in Taraba State

LGA	Number examined	Immuno-fluorescent	
		Negative	Positive
Jalingo	160	135(84.2)	25(15.8)
Kurmi	160	144(90.2)	16(9.8)
Total	320	279(87.2)	41(12.8)

Chi-square=1.012

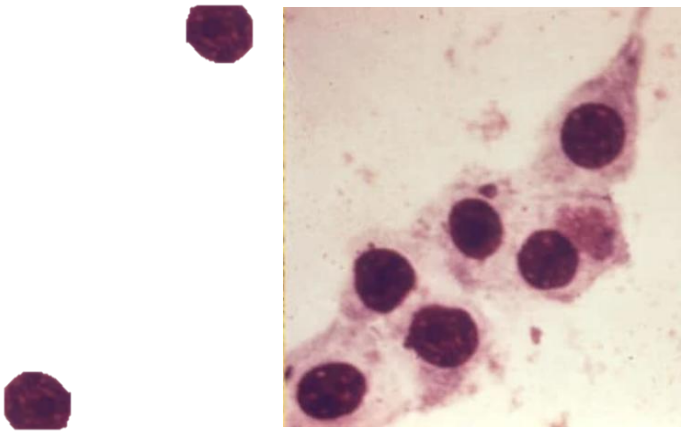


Figure 1: *Chlamydia trachomatis* from a cervicovaginal swab showing intracellular clusters of spore-like elementary bodies within a large epithelial cell.

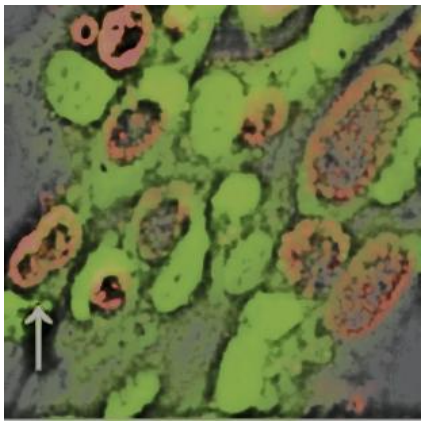


Figure 2: DFA monoclonal antibody that were performed from the stained Pap smear of positive *C. trachomatis*

Molecular identification and characterization of *C. trachomatis* using Polymerase Chain Reaction (PCR)

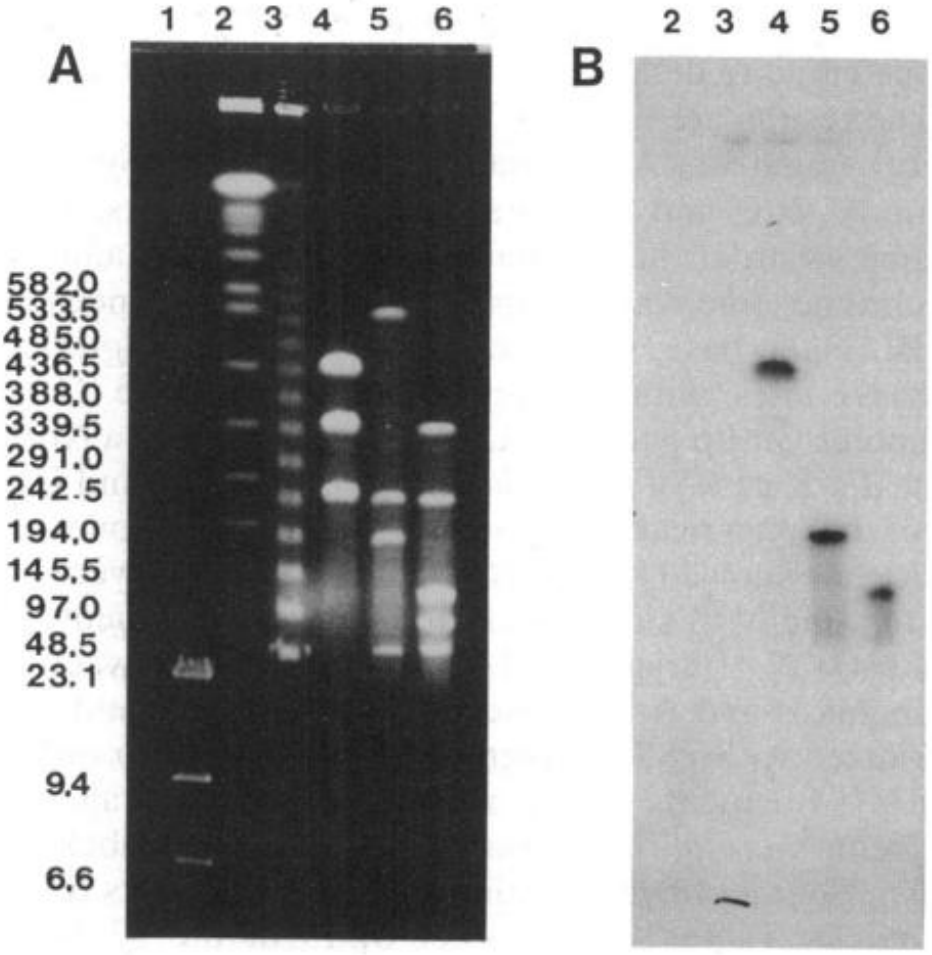


Figure 3: (A) Gel electrophoresis Lanes: M, Molecular ladder, Lanes 4 through 6 are *C. trachomatis* L2 DNA digest with NotI. (B) Autoradiography of the same gel transferred to a nylon membrane and probed with 2P-labeled pG83 encoding gene from *C. trachomatis*

Restriction endonuclease digestion of *C. trachomatis* L2 DNA, as shown in Figure 3, and genome size estimation revealed a G+C content of 45%. To generate manageable restriction fragments, enzymes with 8-bp recognition sequences were used. Double digestion produced six fragments, two of which co-migrated. Fragment sizes ranged from 555bp to 10kb, with optimal separation achieved using a pulse time ramp from 15s to 75s over 20h. Further digestion with Sse8387I (CCTGCAIGG) generated 17 fragments ranging from 220bp to 9kb, with an additional 10 bands between 65kb and 21kb. The larger fragments required a pulse time ramp from 1s to 15s over 20h for optimal separation. The

estimated fragment sizes had a sum variation of less than 2%, resulting in a calculated chromosome size of 1,045kb.

DISCUSSION

Chlamydia trachomatis remains a significant public health concern globally, particularly among women of reproductive age. Its asymptomatic nature often leads to underdiagnosis, contributing to complications such as infertility and ectopic pregnancies (WHO, 2021). An overall prevalence of 12.8% among women was observed in the current study, with a higher rate in Jalingo (15.8%) compared to Kurmi (9.8%). This prevalence is relatively moderate when juxtaposed with similar studies across the country. For instance, a study in Jos reported a prevalence of 56.1% among gynecologic clinic attendees (Ochei *et al.,* 2017), while research in Lagos found a prevalence of 27.7% among women with abnormal cervical smears (Adegbesan-Omilabu *et al.,* 2017). Conversely, lower prevalence rates have been observed in other regions; for example, a study in Zaria reported a 6.7% prevalence among sexually active women (Aliyu *et al.,* 2023). These variations may be attributed to differences in diagnostic methods, study populations, and regional sexual health education levels.

The findings of this study align with the results of other studies on *Chlamydia trachomatis* prevalence and knowledge gaps in similar contexts. A non-significant difference in prevalence between Jalingo and Kurmi was observed ($\chi^2 = 1.012$), which corroborates the findings of Bozicevic *et al*., (2023), Bastidas *et al*., (2023), Huai *et al*., (2020) and O'Connell & Ferone, (2016) that underscore the public health importance of *Chlamydia trachomatis* infections, particularly in Africa. Additionally, the high prevalence among married women (186, 89.9%) reflects the findings of Yunusa *et al*., (2024), Hu *et al*., (2021) and Malhotra *et al*., (2013) which highlighted the association between *Chlamydia trachomatis* and severe genitourinary complications.

The study highlighted limited awareness among participants regarding *C. trachomatis* infection. Only 8.9% of women knew that asymptomatic individuals could transmit the infection. This aligns with findings from Kano, where 95.2% of patients were unaware of *C. trachomatis* and its complications (Nwankwo & Sadiq, 2014). Similarly, in Ogun State, a study reported a 31.7% prevalence among gynecology clinic attendees, with a significant association between infection and lack of condom use (Ajani *et al.,* 2017). These findings

suggest that latent and recurring infections, as well as asymptomatic cases, are major obstacles to controlling this bacterial STI, reinforcing the importance of early screening and treatment to prevent transmission and complications as well as underscoring the need for enhanced sexual health education across the country as earlier reported by Chlebus *et al.* (2024), Aseeri *et al.* (2023), Alshemeili *et al.* (2023) and Adachi *et al.* (2021).

In this study, attitudes toward testing did not significantly correlate with infection status. For instance, women who felt stigmatized or ashamed by the test offer had infection rates similar to those who did not report such feelings. This suggests that emotional responses may not directly influence infection risk but could affect willingness to undergo testing. In contrast, a study in Ilorin and Offa reported that marital status and number of sexual partners were significantly associated with *C. trachomatis* seroprevalence among HIV-positive women (Omosigho *et al.,* 2024).This finding is consistent with the findings of Umar *et al*., (2018) and Ornelas-Eusebio *et al*., (2020) who suggested that *Chlamydia trachomatis* infection can have serious implications if left untreated, especially for women of reproductive age.

The present study found no significant association between reported sexual behaviours and infection rates, except for anal sexual contact, which was associated with a lower infection rate ($p=0.029$). This counterintuitive finding may result from reporting biases or protective behaviours in this subgroup. Other studies have identified multiple sexual partners and lack of condom use as significant risk factors for *C. trachomatis* infection (Ajani *et al.,* 2023). The discrepancy underscores the complexity of accurately assessing transmission dynamics and the influence of self-reported data.

In terms of molecular characterization, the study used immune-fluorescent analysis to assess *Chlamydia trachomatis* infection, finding an overall prevalence rate of 71.7%. The breakdown showed that 22 (71.0%) positive cases came from Jalingo, while 11 (73.3%) came from Kurmi, aligning with the trends observed in the initial prevalence study. Polymerase Chain Reaction (PCR) was used for molecular identification, and the genome of *C. trachomatis* was found to have a plasmid consisting of 1,042,519 nucleotide base pairs, with approximately 894 protein-coding sequences. The genome's G+C content was determined to be 45%, consistent with findings from previous studies and the study's results were corroborated by the work of Reyes-Lacalle *et al.* (2022) and Zhou *et al.* (2024)

who estimated the *C. trachomatis* chromosome size to be around 1,045 kb, which is supported by the current study's finding using restriction endonuclease digestion.

In addition to confirming the presence of the chlamydia genome in the studied samples, the study's restriction enzyme analysis revealed six major fragments after double digestion with enzymes, with sizes ranging from 555 bp to 10 kb. This molecular characterization further supports the understanding of the chlamydia genome, which lacks significant duplications, and reinforces the importance of screening and early diagnosis to prevent the spread of *C. trachomatis*. These findings add to the growing body of knowledge about *C. trachomatis*, which continues to highlight its potential to cause severe genitourinary complications, particularly in women. This also reflects the ongoing challenges in the control of sexually transmitted infections (STIs), as noted by Jones *et al.*, (2020), Sigalova *et al.*, (2019), and Versteeg *et al.*, (2018), who emphasized the need for comprehensive STI control strategies, especially considering the asymptomatic nature of many chlamydia cases.

CONCLUSION

Chlamydia trachomatis remains one of the most widespread bacterial STIs globally and presents a significant public health concern. This study highlights the high prevalence of the infection, particularly in communities with elevated transmission risk factors such as polygamy. It emphasizes the superiority of polymerase chain reaction (PCR) as a diagnostic tool, detecting more positive and suspicious cases than traditional methods like antimicrobial, immunofluorescence, and enzyme assays. Given that up to 70% of infected women are asymptomatic, delayed diagnosis can lead to serious complications including pelvic inflammatory disease, infertility, and ectopic pregnancy. The findings advocate for expanded access to PCR-based testing and routine screening to effectively control the spread and reduce the reproductive health burden of *C. trachomatis*. These findings also suggest the need for targeted interventions such as routine screening in primary healthcare centers, public awareness campaigns, and distribution of free condoms to high-risk populations.

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