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Molecular epidemiology of genital *Chlamydia trachomatis* infections among women of reproductive age living with HIV/AIDS in a Nigerian tertiary health institution

*[1,2,3]Abayomi, S. A., [3]Akanbi, A. A., [2]Taiwo, S. S., [4]Omoare, A. A., and [5]Adegboro, B.

[1]Cancer Research UK, Swansea, Wales, United Kingdom
[2]Department of Medical Microbiology and Parasitology, LAUTECH Teaching Hospital, Ogbomoso, Nigeria
[3]Department of Medical Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria
[4]Public Health Laboratory Services, Nigeria Centre for Disease Control and Prevention (NCDC), Abuja, Nigeria
[5]Department of Medical Microbiology and Immunology, Nile University of Nigeria, Abuja, Nigeria
*Correspondence to: subslaabayomi@gmail.com

Abstract:

Background: *Chlamydia trachomatis* is the most prevalent bacterial pathogen causing sexually transmitted infections (STIs) globally. This study investigates the genovars of genital *C. trachomatis* infection among women of reproductive age living with HIV/AIDS in Ilorin, Nigeria.
Methodology: Endocervical samples were collected from a total of 402 consecutively recruited HIV-infected women attending the highly active anti-retroviral therapy (HAART) clinic of the University of Ilorin Teaching Hospital. Socio-demographic and clinical data of participants were collected through interviewer-administered structured questionnaire. *Chlamydia trachomatis* was detected in the samples by conventional polymerase chain reaction (PCR) amplification of *C. trachomatis* cryptic plasmid, and genotyping was performed using multiplex allele-specific (MAS) PCR to detect serovars D-K and L1-L3. Data were analyzed using SPSS version 20.0 Associations between variables were determined using Chi-square test, with significance set at $p<0.05$.
Results: The overall prevalence of *C. trachomatis* was 18.0%, and highest in women aged 18–25 years (33.3%). Genovars D–K were identified in 43.0% of cases, L1–L3 in 23.6%, and 33.3% could not be genotyped by the method used. Significant risk factors associated with *C. trachomatis* infection included previous STI history ($p=0.000$), non-use of barrier contraceptives ($p=0.009$), and prior STI treatment ($p=0.000$). There was a significant association of *C. trachomatis* infection and HIV viral load ($p=0.041$).
Conclusion: This study provides information on the epidemiological distribution of *C. trachomatis* genovars among women with HIV/AIDS in Ilorin, Nigeria. The genovars D-K and L1-L3 identified are good guide for appropriate management of *C. trachomatis* infections, including complications, contact tracing and prevention.

Keywords: *Chlamydia trachomatis*, HIV/AIDS, genovars, STI, Viral load

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Épidémiologie moléculaire des infections génitales à *Chlamydia trachomatis* chez les femmes en âge de procréer vivant avec le VIH/sida dans un établissement de santé tertiaire Nigérian

*[1,2,3]Abayomi, S. A., [3]Akanbi, A. A., [2]Taiwo, S. S., [4]Omoare, A. A., et [5]Adegboro, B.

[1]Recherche sur le Cancer au Royaume-Uni, Swansea, Pays de Galles, Royaume-Uni
[2]Département de Microbiologie Médicale et Parasitologie, Hôpital Universitaire LAUTECH, Ogbomoso, Nigéria
[3]Département de Microbiologie Médicale et Parasitologie, Hôpital Universitaire d'Ilorin, Ilorin, Nigéria
[4]Services Laboratoire Santé Publique, Centre Nigérian Contrôle et Prévention des Maladies (NCDC), Abuja, Nigéria
[5]Département de Microbiologie Médicale et d'Immunologie, Université du Nil du Nigéria, Abuja, Nigéria
*Correspondance à: subslaabayomi@gmail.com

Résumé:

Contexte: *Chlamydia trachomatis* est l'agent pathogène bactérien responsable d'infections sexuellement transmissibles (IST) le plus répandu dans le monde. Cette étude examine les génovars d'infection génitale à *C. trachomatis* chez des femmes en âge de procréer vivant avec le VIH/sida à Ilorin, au Nigéria.
Méthodologie: Des échantillons endocervicaux ont été prélevés auprès de 402 femmes infectées par le VIH,

recrutées consécutivement et fréquentant la clinique de traitement antirétroviral hautement actif (HAART) du CHU d'Ilorin. Les données sociodémographiques et cliniques des participantes ont été recueillies au moyen d'un questionnaire structuré administré par un intervieweur. *Chlamydia trachomatis* a été détectée dans les échantillons par amplification par réaction en chaîne par polymérase (PCR) conventionnelle du plasmide cryptique de *C. trachomatis*, et le génotypage a été réalisé par PCR multiplex spécifique d'allèle (MAS) pour détecter les sérovars D à K et L1 à L3. Français Les données ont été analysées à l'aide de SPSS version 20.0. Les associations entre les variables ont été déterminées à l'aide du test du Chi carré, avec une signification fixée à $p<0,05$.
Résultats: La prévalence globale de *C. trachomatis* était de 18,0%, et la plus élevée chez les femmes âgées de 18 à 25 ans (33,3 %). Les génovars D à K ont été identifiés dans 43,0% des cas, L1 à L3 dans 23,6%, et 33,3% n'ont pas pu être génotypés par la méthode utilisée. Les facteurs de risque significatifs associés à l'infection à C. trachomatis comprenaient les antécédents d'IST ($p=0,000$), la non-utilisation de contraceptifs barrières ($p=0,009$) et un traitement antérieur contre les IST ($p=0,000$). Il y avait une association significative entre l'infection à *C. trachomatis* et la charge virale du VIH ($p=0,041$).
Conclusion: Cette étude fournit des informations sur la répartition épidémiologique des génovars de *C. trachomatis* chez les femmes vivant avec le VIH/sida à Ilorin, au Nigéria. Les génovars D à K et L1 à L3 identifiés constituent une bonne indication pour la prise en charge appropriée des infections à *C. trachomatis*, notamment en ce qui concerne les complications, la recherche des contacts et la prévention.

Mots-clés: *Chlamydia trachomatis*, VIH/sida, génovars, IST, charge virale

Introduction:

Sexually transmitted infections (STIs) represent not only a public health challenge but also a personal crisis for millions, silently crippling reproductive health worldwide (1,2). Among these, *C. trachomatis* stands out as a bacterial infection with far-reaching consequences, capable of remaining asymptomatic while causing severe reproductive damage (3). The WHO reports over one million new STI cases daily, underscoring the global scale of this issue (1). Among these, *C. trachomatis* has emerged as the most prevalent bacterial STI, with profound implications for reproductive health and fertility. Often asymptomatic, *C. trachomatis* infections can silently progress to severe complications, including pelvic inflammatory disease (PID), ectopic pregnancy, and infertility. These risks are compounded in HIV-positive populations, where co-infections can influence HIV disease progression and complicate clinical management.

Globally, *C. trachomatis* continues to represent a significant burden in various regions. In the United States, *C. trachomatis* is the most reported STI, with over two million cases annually and a higher prevalence in young adults aged 15–24 years (2). In Europe, routine screening programs report a prevalence of 3%–6% among sexually active women, underscoring regional differences in diagnostic and preventive strategies (4). Similarly, in Asia, studies have revealed substantial challenges in diagnosing and managing *C. trachomatis*, with prevalence rates ranging from 5-10% in countries like China and India, particularly among high-risk groups (5).

In sub-Saharan Africa, including Nigeria, the prevalence of *C. trachomatis* infection among reproductive-age women varies widely, ranging from 6% to 40%, with significant socioeconomic and healthcare disparities contributing to this variation (6). This aligns with studies in similar Nigerian settings, where *C. trachomatis* infection has been linked to poor reproductive outcomes and an increased risk of HIV transmission. Despite these alarming statistics, limited research has explored the molecular epidemiology of *C. trachomatis* in resource-limited settings like Nigeria. Addressing this gap is critical for improving screening strategies and ensuring tailored interventions. This study aims to fill this critical gap by investigating the molecular epidemiology of *C. trachomatis* among HIV-positive women in Ilorin, Nigeria.

Materials and method:

Study area, design and ethical approval:
This was a hospital-based descriptive cross-sectional study of HIV-infected women aged 18 to 49 years attending the highly active antiretroviral therapy (HAART) clinic of the University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria. UITH is a healthcare institution with a capacity of 600 beds, which additionally operates as a referral center for residents of Kwara and the adjacent States of Niger, Ekiti, Osun, Oyo, and Ondo. Geographically, it is situated at a latitude of 8.3ºN and a longitude of 4.3ºE in the northcentral region of Nigeria.

The mean frequency of patient visits for HAART treatment is approximately 6000 monthly consultations (7). The Institutional Review Board (IRB) of UITH approved the conduct of the study with approval number UITH/CAT/189/19B/195. At recruitment, each participant provided informed consent to participate in the study.

Sample size and participant selection:
The sample size of 402 patients was calculated using the modified Fisher's formula for proportions with population less than 10000 (8), with an estimated prevalence rate set at 50% and adjusting for 10% attrition. The patients who satisfied the inclusion criteria were recruited consecutively using the simple random sampling technique until the sample size of 402 was attained over a period of 3 months.

Data, sample collection and transportation:

A structured questionnaire was designed and interviewer-administered to collect relevant demographic and clinical information including age, marital status, educational level, relevant symptoms of sexually transmitted infections such as history of vaginal discharge and lower abdominal pain.

Endocervical swab was collected aseptically from all participants at the HAART clinic by one of the researchers (ASA). A Cusco's speculum was inserted into the vagina to expose the cervix while the patient was lying supine with the legs supported with a stirrup. Endocervical swab was taken by inserting a cytobrush into the endocervical canal, followed by a gentle 360º rotation of the cytobrush 5 times. The cytobrush was withdrawn carefully to avoid contact with the vaginal mucosa and then placed inside a tube containing a liquid-based cytology (Eziprep™) preservative, stored at 2-8°C, and transported to the laboratory for genomic DNA extraction.

Five millilitres (5 ml) of venous blood was also collected from each participant into ethylene diamine tetra acetic acid (EDTA) bottle by aseptic venepuncture, for HIV testing.

Detection of HIV and viral load estimation:

Presumptive detection of HIV was done using Determine HIV 1/2 Ag/Ab (Abbot) kit. A Pasteur pipette was used to place a drop of blood onto the absorbent pad on the strip of the kit. A drop of chase buffer was added to the specimen pad and the result was interpreted after 15 minutes and recorded. A reactive result was interpreted by two lines appearing in both control and test areas of the strip.

Confirmatory HIV detection was performed using UniGold™ (Trinity Biotech) strip, a rapid immunoassay for qualitative detection of antibodies to HIV-1 and HIV-2 in the blood. Two drops of blood were applied to the same port, followed by two drops of wash solution and allowed to react for 15 minutes. A reactive result is interpreted by a line at the test and another one at the control region of the test device.

The results of the viral load of each HIV-infected participant performed by real-time (rt) PCR at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria, in the 3 months preceding enrolment were retrieved from the laboratory records of each participant.

DNA extraction:

Chlamydia trachomatis DNA was extracted from each endocervical swab sample using a commercially available DNA extraction kit (FavorPrep™Ping Tong, Taiwan) according to the manufacturer's instructions. Eluted total DNA was subsequently stored at -20℃ for PCR analysis.

Polymerase chain reaction amplification detection of *C. trachomatis* cryptic plasmid:

Conventional PCR as described by Cufinni and co-workers (9) was used to amplify the cryptic plasmid DNA (usually present in all *C. trachomatis* strains) in all the endocervical samples from which DNA has been extracted. The appropriate volumes of Master Mix (made up of thermostable Taq DNA polymerase, $MgCl_2$, nucleotide triphosphates, reaction buffer), forward (5'-AGTATTTCCGGAGCAGGC-3') and reverse (5'-TTACCTCGAAAAACTGTTCC-3') primer, template genomic DNA and distilled water were dispensed into each PCR tube and the mixture vortexed.

Amplification was done in a thermal cycler (Eppendorf) programmed with 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 60 seconds, extension at 72°C for 90 seconds, and final extension at 72°C for 5 minutes. Positive control (*C. trachomatis* reference serovar-L2 strain obtained from National Microbiology Laboratory, Winnipeg, Canada), and negative controls (distilled water) were also processed using the same protocol.

The tubes were removed from the thermal cycler after the reaction has been completed and the amplicons were resolved on electrophoresis in 1.5% agarose gel containing a gel stain dye (10µl SYBR) safe to produce bands that were visualized in a gel documentation system with an ultraviolet trans-illuminator and a documentation computer system. The 100 base pair molecular weight standard DNA marker was used to determine the size of the amplified PCR product. A positive reaction for *C. trachomatis* was confirmed by the presence of a band corresponding to 201 amplicon size.

Multiplex allele specific PCR (MAS-PCR) for *C. trachomatis* serovar identification:

The multiplex allele-specific PCR (MAS-PCR) for *C. trachomatis* designed by Cai and colleagues (10), was used to identify the serovars of *C. trachomatis* in all the samples that amplified with the conventional PCR for cryptic plasmid, and categorized them into serovars D-K, L1-L3 or untyped strain.

The MAS-PCR is based on the variation in the sequence of the polymorphic membrane protein H (*pmpH*) gene of *C. trachomatis* (which has a unique 36-nucleotide base deletion compared with the other *C. trachomatis* serovars) for which primers that can amplify the unique specific sequences in a multiplex PCR system to distinguish serovars A-C, D-K and L1-L3 have been developed.

The reagents used for MAS-PCR includes ready to use Master Mix (5µl), 0.5µl of outer primers (*pmph*-F and *pmph*-R), 0.5µl of inner primers (*pmph*-R2-LGV and *pmph*-R3-CT) (Macrogen, Europe), 17.5µl of distilled

water and 1.5µl of template DNA. Two sets of MAS-PCR were done with two primers in combination of each set; set one with *pmpH*-F (forward outer primer), *pmph*-R (reverse outer primer) and *pmpH*-R2-LGV (inner primer) for *C. trachomatis* serovars L1, L2 or L3; and set two with *pmpH*-F (forward outer primer), *pmpH*-R (reverse outer primer) and *pmpH*-R3-CT (inner primer) for *C. trachomatis* serovars D-K (Table 1).

After an initial denaturation step at 95ºC for 15 minutes, the following amplification conditions were utilized; denaturation at 94ºC for 30 seconds, annealing at 55ºC for 30 seconds and extension at 72ºC for 30 seconds, followed by 25 cycles at 94ºC for 30 seconds, 53ºC for 40 seconds and 72ºC for 40 seconds, with a final extension at 72ºC for 2 minutes. A non-template control containing sterile distilled water was included in each experimental run. Amplified products were electrophoresed on 2% agarose gel and visualised under ultraviolet light and photographed with a gel documentation system.

The set of *pmph*-F, *pmph*-R and *pmph*-R3-CT that positively amplified is expected to produce two bands of 224 bp and 122 bp sizes to indicate *C. trachomatis* serovars D-K while the set of *pmph*-F, *pmph*-R and *pmph*-R2-LGV that positively amplified is expected to produce two bands of 224 bp and 106 bp sizes to indicate *C. trachomatis* serovars L1, L2 or L3.

Course of treatment:

Participants who tested positive for *C. trachomatis* were treated with doxycycline or azithromycin based on the National STI guidelines. Counselling was offered to promote adherence, educate on safe sexual practices, and address stigma associated with STIs and HIV. Follow-up appointments were scheduled to monitor treatment outcomes and reinforce behavioral changes aimed at reducing reinfection risk.

Statistical analysis:

Data were analyzed using SPSS version 20.0. Associations between variables were assessed using Chi-square test, with significance set at $p<0.05$.

Results:

Prevalence of *Chlamydia trachomatis* infection*:*

The overall prevalence of *C. trachomatis* infection was 18.0% (n=72/402). Women aged 18–25 years had the highest prevalence (33.3%, n=1/3), followed by age group 46–49 years (22.0%, n=22/100), and 31–40 years (17.8%, n=24/135) (Table 1), but there was no significant association of *C. trachomatis* infection with age ($p=0.5656$).

However, there was significant association of *C. trachomatis* infection with marital status ($p=0.0014$), with higher prevalence in divorced (50.0%, n=1/2) and married (23.0%, n=63/274) women compared to other women, but no significant association with educational status of the participants ($p=0.2156$) (Table 2).

Symptoms associated with *C. trachomatis* infection:

Among 72 women positive for *C. trachomatis*, 72.2% (n=52) reported vaginal discharge, compared to 43.0% (n=142) among 330 women negative for *C. trachomatis* infection ($p=0.000$). Other symptoms significantly more frequent in women with *C. trachomatis* infections are pain during sexual intercourse ($p=0.048$), bleeding after sexual intercourse ($p=0.004$), bleeding between periods ($p=0.019$) and history of STIs ($p=0.000$) (Table 3).

Table 1: Primers and conditions of the multiplex allele-specific PCR for *Chlamydia trachomatis* genotyping

Target gene	Primer sequence	Amplicon size	Melting point	Specificity	Reference
pmpH	Outer primers- *pmph*-F-AGTATTTCCGGAGCAGGC *pmph*-R-TTACCTCGAAAAACTGTTCC	224 bp or 260 bp	55ºC	*C. trachomatis*	10
pmpH	Inner primers- *pmph*-R2-LGV–TAACTGTTGGAGCAGGCG *pmph*-R3-CT- CTTGAAGCAGCAGGAGCT	106 bp 122 bp	52ºC 52ºC	Serovar L1-L3 Serovar D-K	10

Table 2: Socio-demographic characteristics of HIV-infected women with *Chlamydia trachomatis* infections in Ilorin, Nigeria

Characteristic	No positive for *C. trachomatis* (n=72, 18.0%)	No negative for *C. trachomatis* (n=330, 82.0%)	Chi square (x^2)	*p* value
Age group (years)				
18-25	1 (33.3)	2 (66.7)	2.954	0.5656
26-30	2 (9.5)	19 (90.5)		
31-40	24 (17.8)	111 (82.2)		
41-45	23 (16.1)	120 (83.9)		
46-49	22 (22.0)	78 (78.0)		
Marital status				
Single	2 (7.7)	24 (92.3)		
Married	63 (23.0)	211 (77.0)		
Widowed	4 (6.3)	59 (93.7)		
Separated	2 (5.4)	35 (94.6)		
Divorced	1 (50.0)	1 (50.0)	17.723	0.0014*
Educational status				
None	7 (16.3)	36 (83.7)		
Primary	15 (19.0)	64 (81.0)		
Secondary	17 (12.9)	115 (87.1)		
Tertiary	33 (22.4)	114 (77.6)	4.463	0.2156

* = statistically significant

Table 3: Comparative analysis of clinical symptoms between HIV-infected women with and without *Chlamydia trachomatis* infections in Ilorin, Nigeria

Clinical symptom	No positive for *C. trachomatis* (%) (n=72)	No negative for *C. trachomatis* (%) (n=330)	Chi square (x^2)	*p* value
History of vaginal discharge	52 (72.2)	142 (43.0)	20.171	0.000*
History of blisters and spots	2 (2.8)	2 (0.6)	2.803	0.150
History of lower abdominal pain	16 (22.2)	48 (14.5)	2.602	0.079
Pain during sexual intercourse	8 (11.1)	19 (5.8)	4.033	0.048*
Bleeding after sexual intercourse	4 (5.6)	1 (0.3)	13.275	0.004*
Bleeding between periods	3 (4.2)	1 (0.3)	8.956	0.019*
History of pain on passing urine	4 (5.6)	12 (3.6)	0.570	0.317
Previous STI	53 (73.6)	140 (42.4)	23.031	0.000*

* = statistically significant; STI=Sexually transmitted infection

Risk factors associated with *C. trachomatis* infection:

Risk factors associated with *C. trachomatis* infection included age at first intercourse (p=0.000), history of previous STI (p=0.000), use of protection against STIs (p=0.009), types of protection against STIs (p=0.000), and prior STD treatment (p=0.000) (Table 3).

Frequency distribution of *C. trachomatis* genovars:

Of the 72 women with *C. trachomatis* infection, as confirmed by PCR amplification of the cryptic plasmid (Fig 1), 31 (43.0%) were genovars D-K (Fig 2), 17 (23.6%) were genovars L1-L3 (Fig 3) while 24 (33.3%) could not be genotyped by the method used.

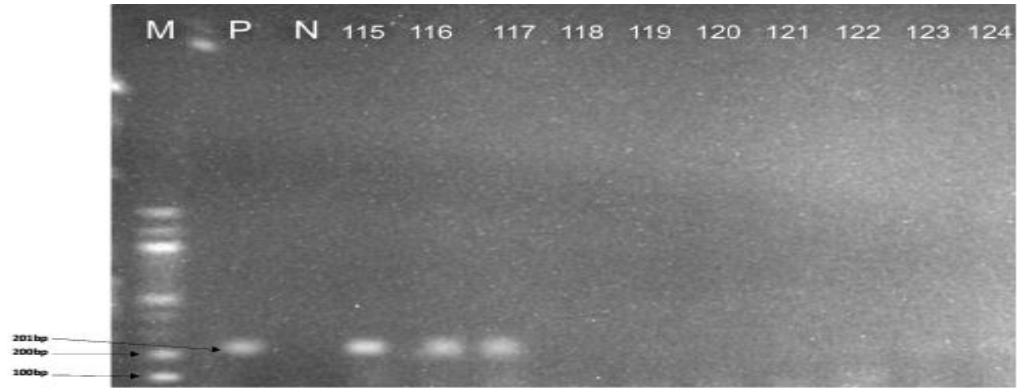
HIV viral load and *C. trachomatis* infection:

HIV load was significantly associated with *C. trachomatis* infection (p=0.0419). HIV loads of 101-1000 and >1000 copies/µl were significantly higher in women with *C. trachomatis* infections (11.3% and 16.1% respectively) compared to women without *C. trachomatis* infection (8.9% and 14.2% respectively) (Table 4).

Table 4: Risk factors of *Chlamydia trachomatis* in HIV infected women in Ilorin, Nigeria

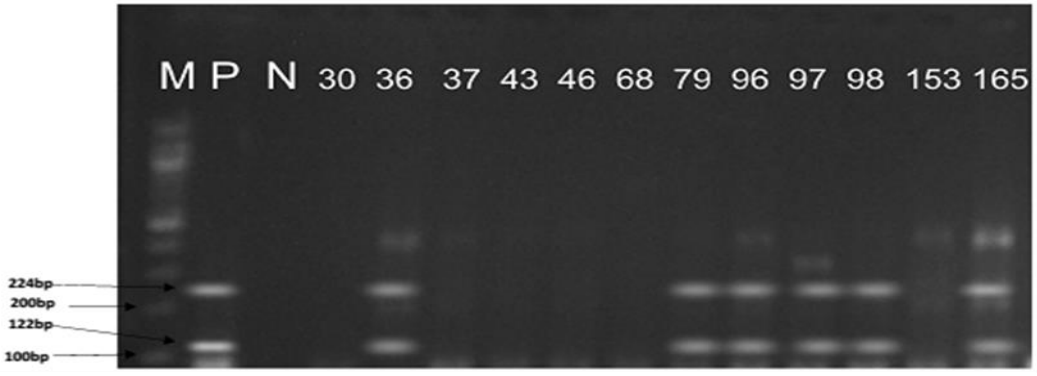
Risk factor	No positive for *C. trachomatis* (n=72, 18.0%)	No negative for *C. trachomatis* (n=330, 82.0%)	Chi square (x^2)	*p*-value
Age at first intercourse (years)				
18-25	20 (25.0)	60 (75.0)		
26-49	52 (16.6)	262 (83.4)	18.501	0.000*
Number of sexual partners				
None	4 (7.4)	50 (92.6)		
1	68 (19.7)	278 (80.3)		
>1	0	1 (100.0)	5.301	0.064
Use of protection against STI				
Yes	27 (13.2)	177 (86.8)		
No	45 (62.2)	152 (46.2)	6.279	0.009*
History of previous STI				
Yes	53 (26.9)	140 (73.1)		
No	19 (9.1)	190 (90.9)	23.031	0.000*
Type of protection against STI				
None	27 (13.3)	176 (86.7)		
Condom	40 (20.8)	152 (79.2)		
Others	5 (100.0)	0	31.804	0.000*
Treated for STI				
Yes	45 (23.4)	147 (76.6)		
No	3 (75.0)	1 (25.0)	32.94	0.000*

* = statistically significant; STI=Sexually transmitted infection



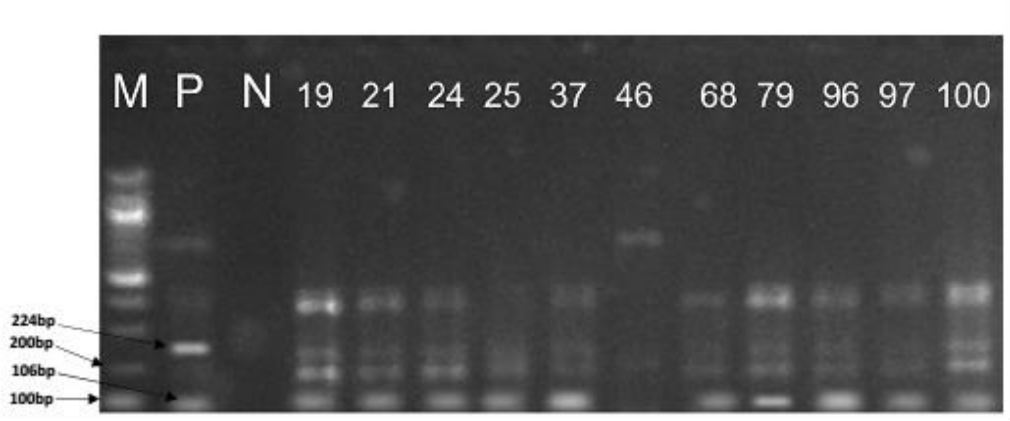
M= Molecular ladder; P = Positive Control; N = Negative Control; Positive samples 115, 116 and 117 amplified at 201bp for *C. trachomatis*; Negative samples 118-124 did not amplify

Fig 1: Gel electrophoresis of representative *Chlamydia trachomatis* showing amplicon size of 201 bp for cryptic plasmid



M= Molecular ladder; P = Positive Control; N = Negative Control; Positive samples 36, 79, 96, 97, 98, 165 amplified at 224bp for *C. trachomatis;* Negative samples 30, 37, 43, 46, 68 did not amplify

Fig 2: Gel electrophoresis of representative *Chlamydia trachomatis* showing amplicon size 224 bp (*pmph* gene with outer primer) and another amplicon size 122 bp ((*pmph* gene with inner primer) for genovars D-K



M= Molecular ladder; P = Positive Control; N = Negative Control; Positive samples 19, 21, 24, 25, 37, 68, 79, 96, 97, 100 amplified at 224bp for *C. trachomatis* and 106 bp for *C. trachomatis* genovars L1-L3

Fig 3: Gel electrophoresis of representative *Chlamydia trachomatis* showing amplicon size 224bp (*pmph* gene with outer primer) and another amplicon at 106 bp (*pmph* gene with outer primer) for genovars L1-L3

Table 5: Comparison analysis of viral load of HIV infected women with and without *Chlamydia trachomatis* infection in Ilorin, Nigeria

Viral load (copies/µL)	No positive for *C. trachomatis* (%) (n=61)	No negative for *C. trachomatis* (%) (n=283)	Chi square (x^2)	*p* value
0	2 (3.2)	0	9.913	0.0419*
<20	33 (53.2)	167 (59.2)		
20-100	10 (16.1)	50 (17.7)		
101-1000	7 (11.3)	25 (8.9)		
>1000	9 (16.1)	41 (14.2)		

n = number of participants whose viral loads were available; * = statistically significant

Discussion:

Chlamydia trachomatis has been identified as an accelerator in the transmission and acquisition of HIV (11). Most of the women do not experience symptoms, however testing them helps to identify this infection. In this present study, 402 endocervical samples were collected and *C. trachomatis* was detected using conventional PCR technique. The overall prevalence of 18% is similar to the prevalence reported by Spinillo et al., (12) of 18.3% in HIV infected women, a study which was conducted in Italy. This is however lower than the prevalence reported in South Africa where a value of 39.2% obtained in HIV infected women (6). In Nigeria however, studies in various cities produce divergent reports of *C. trachomatis* prevalence. A study in Lagos reported a prevalence of 51% among pregnant and non-pregnant women (13), while Oloyede et al*.,* (14) reported a prevalence of 18.2% also in Lagos, Nigeria. Mawak et al., (15) reported a prevalence of 30.2%, while Ikeme et al., (16) in Enugu reported a prevalence of 29.4%. These variations may be due to differences in the population studied and laboratory detection methods used.

The age group with the highest prevalence of *C. trachomatis* infection in our study is 18-25 years (33.3%, n=1/3), which agrees with the study that reported younger age group < 25 years to be at greater risk of *C. trachomatis* infection because of the not yet fully developed cervix, which increase their susceptibility to infection (17). A previous study also reported that *C. trachomatis* infection is most common in people aged less than 25 years due to greater exposure to high-risk sexual behaviours (18). However, other studies in Africa have reported higher prevalence of 35.6% in women aged 25-45 years, and a study in Mpumalanga Province reported high prevalence of 52.8% in female miners aged 46-49 years (6).

Age at first intercourse is one of the risk factors of *C. trachomatis* infection. This is the case in our study where women who had their first sexual intercourse between age 18 and 25 years had significantly higher prevalence of *C. trachomatis i*nfection of 25.0% compared to women who had their first intercourse between age 26 and 49 years, with prevalence of 16.6% (p=0.000), but disagrees with the study by Mafokwane et al., (6) who reported higher prevalence of 35.6% among women aged 25–45 years (6), and a previous study in southeast Nigeria that reported higher prevalence of 43.9% among women between the age of 26–30 years attending gynaecological clinics (13).

Previous studies have also reported

that unprotected sex is one of the major risk factors for acquisition of sexually transmitted infections. Although there was a significant association between use of protection and *C. trachomatis* infection in our study (p=0.000), the prevalence of infection was highest among those who used unknown means of protection (100%, n=5/5), followed by those who used condoms (20.8%, n=40/192) and lowest among those who did not use any protection (13.3%, n=27/203). This contradicts the study by Dubbink et al., (19) who reported a high infection prevalence of 40% in people who did not use condoms. However, our study findings may indicate that the use of condom may not protect against STDs, which may likely be due to inappropriate use of condoms during sexual intercourse in our participants, while those using unapproved or unvalidated means of protection are at high risk of *C. trachomatis* infection.

Of the *C. trachomatis* detected in the 72 infected women, only 48 could be genotyped by the MAS-PCR method used, with genovars D-K identified in 31 (43.0%) and genovars L1-L3 in 17 (23.6%) cases. This is similar to a study by Lesiak-Markowicz et al., (20) who identified and reported serovars D-F as the most recognised serovars worldwide. Foschi et al., (4) also identified serovars D-K as the most prevalent serovar in their study where they reported an overall prevalence of 83%. Our inability to type the remaining 24 *C. trachomatis* by MAS-PCR assay may have been due to low bacterial load in the endocervical specimens and technical limitations of multiplex PCR.

Although there were 17 *C. trachomatis* genovars L1-L3 in our study, only 2 of the 72 (2.8%) women with *C. trachomatis* infection had history of vaginal sores and blisters, indicating that most of the women with genovars L1-L3 infection are asymptomatic. This is similar to the report of a study by Haro-cruz et al., (5) on endocervical samples of infertile women in Mexico, where genovar L2 strain was found in (8.7%) of the infertile women and these women did not present with or had history suggestive of a sexually transmitted infection or ulcers or sores at the time of specimen collection.

Lymphogranuloma venereum (LGV) is a sexually transmitted infection caused by serovars L1, L2 and L3 of *C. trachomatis*. It is endemic in Africa, India, South America, Southeast Asia and the Caribbean. Studies have reported that women are more likely to become infected with LGV (21). The classical picture of LGV (buboes and lymphadenopathy) was not present in the women that had L1-L3 genovars in our study, which may mean that they were most likely asymptomatic. Gomes et al., (21) reported the detection of 7 LGV from specimens collected from men and women who had no clear LGV symptoms. Another explanation for this may be that point mutations are present in the genome of L2 variants that prevent the development of symptoms of LGV or proctitis (21).

In agreement with our study and others in the literatures (13,21) in relation to the symptomatic course of most *C. trachomatis* infection, a proportion of HIV seropositive women do not have any symptom or sign of infection on physical examination. Many cases without *C. trachomatis* infection in our study also presented with symptoms such as lower abdominal pain, dysuria, dyspareunia and vaginal discharge. This may be due to the presence of possible other STD pathogens aside *C. trachomatis* such as *Ureaplasma urealyticum*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Candida albicans* and others. This will require further molecular epidemiological study of these other STD pathogens.

Conclusion:

Our study provides information on the prevalence, risk factors and epidemiological distribution of genovars of *C. trachomatis* infection among women with HI`V/AIDS in Ilorin, Nigeria using MAS-PCR assay. The genovars D-K and L1-L3 identified are good guide for appropriate management of *C. trachomatis* infections, including complications, contact tracing and prevention.

Contributions of authors:

ASA was involved in the conceptualization of study, data and sample collection, coordinated the research work, ensured compliance with ethical standards and study protocols, and secured funding through grants. AAA reviewed the literature. TSS developed the methodology used in the study and reviewed the literature. OAA contributed to the optimisation of research protocol, literature review and drafting of initial manuscript. AB reviewed the literature. All authors approved the manuscript submitted for publication.

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Conflict of interest:

Authors declare no conflict of interest

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