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HIGH PREVALENCE OF VAGINAL TRICHOMONIASIS AND GENOTYPE CHARACTERISATION (*T. VAGINALIS* G3) IN PREGNANT WOMEN IN EKITI STATE, NIGERIA

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Abstract
Trichomoniasis, caused by Trichomonas vaginalis, remains the most prevalent curable sexually transmitted infection globally and is associated with adverse reproductive outcomes, particularly during pregnancy. Despite its public health significance, diagnostic gaps, asymptomatic carriage, and limited molecular epidemiological data continue to impede effective control in Nigeria. This study investigated the prevalence and genotype characteristics of T. vaginalis among pregnant women attending antenatal care in Ekiti State. A cross-sectional study was conducted among 220 consenting pregnant women attending the Ekiti State University Teaching Hospital, Ado-Ekiti. Demographic and obstetric information was collected using a structured questionnaire. Participants were aged 21–40 years, predominantly married, educated, and in the third trimester. High vaginal swab samples were examined using wet mount microscopy and Giemsa-stained preparations. Molecular analysis was performed using PCR targeting the T. vaginalis-specific TVSK gene, followed by sequencing, BLAST alignment, and phylogenetic analysis. Data were analysed using SPSS version 23, with statistical significance set at $p \leq 0.05$. The overall prevalence of T. vaginalis gestational infection by microscopy was 33.3%, indicating a high infection burden. A significant association was recorded between age and infection status ($p = 0.047$), with the highest prevalence observed in second-trimester women. No significant association was found with age, marital status, education, parity, or clinical symptoms. Molecular testing confirmed T. vaginalis in four of five microscopy-positive samples and additionally detected one microscopy-negative infection, demonstrating the increased diagnostic power of PCR. Sequencing and phylogenetic comparison revealed that the isolates aligned with T. vaginalis genotype G3 but exhibited distinct nucleotide variations, suggesting localised genetic diversification. The documented high prevalence of trichomoniasis among pregnant women demonstrates a continuing public health challenge in the study area, with potential implications for adverse maternal-neonatal outcomes. The findings support the need for routine antenatal screening, the integration of nucleic acid amplification tests, effective partner management strategies, and enhanced health education. Continued molecular surveillance is recommended to monitor genotype evolution and its possible relationship to virulence or treatment response.

Keywords: *Trichomonas vaginalis*, pregnancy, PCR, genotype G3, antenatal screening, molecular epidemiology.

Introduction
Trichomonas vaginalis is a highly successful, anaerobic, flagellate protozoan parasite and the causative agent of trichomoniasis, recognised as the most prevalent curable sexually transmitted infection (STI) worldwide. Identified first by Alfred François Donne in 1836, the parasite's clinical significance has grown, solidifying its role as a leading cause of non-viral sexually transmitted morbidity (Inusa *et al.,* 2018).

The global disease burden of trichomoniasis is staggering. While historical estimates placed the annual incidence at over 270 million cases (Salakos et al., 2018), current global health monitoring reflects that approximately 156 million new cases arise each year among women and men worldwide. This figure consistently surpasses the combined incidence of chlamydia and gonorrhoea (Rowley *et al*., 2019). This immense scale highlights an urgent need for global public health intervention, particularly as the burden is disproportionately borne by low- and middle-income nations, with high prevalence rates continuously reported across Africa (Sherrard *et al*., 2022). For example, antenatal prevalence in some sub-Saharan African regions has been documented as high as 52–61% (Damali *et al*., 2021), illustrating the concentrated epidemic in these vulnerable communities.

Transmission is fundamentally linked to sexual contact, yet its incidence is profoundly influenced by broader socio-demographic and behavioural factors, including poor personal hygiene, sexual intercourse with multiple partners,

and low socio-economic status (Menezes *et al*., 2016). Recent studies emphasise the concentration of infection within marginalised groups, including incarcerated women, female sex workers, and individuals with HIV, underscoring systemic health equity gaps in screening and access to care (Cunningham *et al*., 2023).

A critical challenge in controlling trichomoniasis is its often-insidious symptomatology. A significant majority of cases, up to 85% in women and 70% in men, remain asymptomatic, facilitating unchecked propagation within sexual networks and allowing the infection to persist for extended periods (Bachmann *et al*., 2000; Sutton *et al*., 2007).

In symptomatic women, the infection typically presents a profuse, often characteristic frothy, foul-smelling vaginal discharge, accompanied by dysuria, vulvo-vaginal irritation, and lower abdominal pain (Oyetunde *et al*., 2016). Pathogenetically, the parasite's presence disrupts the local microenvironment, commonly causing the vaginal pH to shift from its protective acidic range (3.8–4.2) to a more neutral, growth-permissive range (5.0–6.0) (Inusa *et al.,* 2018). Recent molecular research continues to explore the complex adhesion mechanisms used by the parasite that enhance its virulence and colonisation, identifying key surface proteins crucial for host cell interaction (Heine et al., 2024).

In men, the infection frequently manifests as non-gonococcal urethritis, chronic prostatitis, or can contribute to reversible infertility (Squire *et al*., 2020). Beyond its direct effects, *T. vaginalis* can act as a pathogen vector, capable of carrying other organisms attached to its surface into the upper reproductive tract, potentially exacerbating complex inflammatory diseases (Inusa *et al*., 2018).

Trichomonas vaginalis infection is increasingly recognised as a major public health threat because of its link with serious long-term consequences, especially related to reproductive health and HIV transmission (WHO & CDC, 2025). Persistent, untreated trichomoniasis is a confirmed co-factor in the HIV epidemic. The resulting genital tract inflammation and mucosal disruption substantially raise the risk of HIV acquisition and transmission by two to three times (Kissinger, 2015). Therefore, tackling this protozoan infection is a crucial part of integrated HIV prevention strategies.

The impact on maternal and neonatal health is a major concern. The infection is robustly linked to significant adverse birth outcomes, including premature rupture of membranes, preterm delivery, and the delivery of low-birth-weight infants (Hobbs *et al*., 2019). The biological plausibility and consistent findings across recent meta-analyses solidify the causative relationship between antenatal trichomoniasis and poor birth outcomes (Guglielmino & Al-Khalili, 2025). Furthermore, infections increase the risk of postpartum complications, such as upper reproductive tract infections post-caesarean section (Oyetunde *et al*., 2016). In non-pregnant women, unchecked infections can ascend, triggering systemic inflammation and placing patients at risk for atypical Pelvic Inflammatory Disease (PID), chronic pelvic pain, and infertility (Cherpes *et al.,* 2022).

Effective control hinges on overcoming the diagnostic challenges presented by the high rate of asymptomatic carriage. Nucleic Acid Amplification Tests (NAAT) have fully emerged as the gold standard for diagnosis due to their exceptional sensitivity and specificity, far surpassing traditional wet-mount microscopy (Kissinger & Muzny, 2024). Expanding the use of NAAT is paramount for accurate case finding, especially in antenatal and high-risk screening programs. Current public health recommendations emphasise embedding regular NAAT screening within routine women's health visits to enhance detection and curb community transmission (Stenger *et al*., 2022).

The treatment remains centred on the nitroimidazole class of drugs, primarily Metronidazole and Tinidazole (Mabaso & Abbai, 2021). Timely treatment is crucial, not only for patient health but also to immediately mitigate HIV transmission risk. The rising awareness of drug resistance also necessitates ongoing surveillance and adherence monitoring. The high rate of reinfection—often due to untreated partners—demands renewed focus on partner management. Expedited Partner Therapy (EPT), or patient-delivered partner treatment (PDPT), is a highly effective, though underutilised, intervention shown to reduce reinfection rates significantly (Hobbs & Sherrard, 2023). Public health strategy must concentrate on: (1) Expanding access to EPT for vulnerable patients, (2) Reinforcing comprehensive safer sexual practices, and (3) Addressing structural barriers (stigma, healthcare access) that perpetuate high infection rates in marginalised communities (Hobbs *et al.,* 2022).

The gap in the literature addressed by this study lies in the limited data on the prevalence, molecular characterisation, and circulating genotypes of *Trichomonas vaginalis* among pregnant women in Ekiti State, Nigeria. While prior studies reported trichomoniasis prevalence in broader Nigerian populations, few have combined microscopic diagnosis, PCR confirmation, and genotype analysis (specifically G3 strain identification) in antenatal settings.

Methodology

Study Area and Population

This study was conducted at Ekiti State University Teaching Hospital (EKSUTH), located in Ado-Ekiti, Ekiti State, Nigeria. The institution serves as a major referral centre for obstetric and gynaecological services in southwestern Nigeria. The research was carried out over six months, from March to August 2023 and targeted pregnant women attending routine antenatal care at the Obstetrics and Gynaecology Department. A total of 213 participants were

enrolled based on sample size estimation and eligibility criteria. The recruitment of pregnant women at different trimesters ensured a representative assessment of *Trichomonas vaginalis* infection among antenatal attendees within the study period.

Ethical Considerations

Before participant enrolment, ethical approval was obtained from the Research Ethics Committee of EKSUTH (Approval No. SEKSUTH/A67/2023/06/006). All procedures adhered to international biomedical research ethics, including respect for autonomy, confidentiality, beneficence, and non-maleficence (World Medical Association, 2013). Participants were fully informed about the study objectives and procedures before granting written consent.

Sample Size Determination

The minimum sample size (n) was determined using Fisher's formula for prevalence studies. A previous prevalence rate of 16.6% for *T. vaginalis* among pregnant women in Nigeria (Olusola et al., 2019) was used as the estimated proportion (p), while the confidence level (Z) and precision (d) were set at 95% (1.96) and 5% (0.05), respectively. The computation yielded a sample size of 212.72, approximated to 213 participants. This ensured adequate statistical power and minimised sampling error in line with standard epidemiological recommendations (Naing *et al.,* 2006).

Study Design, Inclusion and Exclusion Criteria

A cross-sectional hospital-based design was adopted, which is suitable for estimating point prevalence and associated risk factors of infectious diseases (Setia, 2016). Eligible participants included all pregnant women attending antenatal care at EKSUTH regardless of gestational age. Women who declined consent or had documented cognitive impairment were excluded.

Data and Specimen Collection

Sociodemographic and obstetric data were collected using a structured interviewer-administered questionnaire adapted from validated tools used in similar studies (Ogunniran, 2018). High vaginal swabs were collected aseptically using sterile disposable specula and swab sticks by trained health personnel. Samples were immediately stored in ice-packed containers and transported to Afe Babalola University Biotechnology Centre for laboratory investigations to preserve parasite viability and DNA integrity.

Laboratory Diagnosis and Molecular Procedures

Initial parasitological diagnosis was performed using wet mount microscopy and Giemsa staining to detect motile trophozoites characterised by ovoid shape and jerky motility (Orpin et al., 2023; Marwa et al., 2022). Genomic DNA extraction was performed using the Quick-DNA Universal Kit (Zymo Research), following the manufacturer's protocols. Molecular confirmation was achieved using polymerase chain reaction (PCR) targeting *T. vaginalis*–specific primers as described by Mohammed *et al*. (2023). DNA amplification was carried out using primers: TVSK (forward; 5-ATTGTCGACATTGGTCTTACCCTC-3) and TVSK (reverse;5-TCTGTGCCGTCTTACCTC-3). Amplicons were visualised using 1.5% agarose gel electrophoresis, stained with ethidium bromide. Purified PCR products underwent Sanger sequencing, and phylogenetic analysis was conducted using BioEdit and MEGA11 software.

Statistical Analysis

Data were analysed using SPSS version 23. Descriptive statistics, one-way ANOVA, and Pearson correlation were performed, and significance was set at $p \leq 0.05$.

Results

A total of 220 pregnant women attending the antenatal clinic at Ekiti State University Teaching Hospital, Ado-Ekiti, were examined for *Trichomonas vaginalis* infection. The respondents were aged between 21 and 40 years, with a mean age of 28.3 ± 10.0 years and the modal age group of 31-35 years. All subjects had at least secondary education, and most were married (98.3%) and living in monogamous homes (96.7%). More than half were self-employed (59.3%), and 56.1% had at least one previous childbirth. The majority of participants (67%) were in their third trimester at the time of sampling.

With respect to obstetric and gynaecological presentation, 43.9% reported vaginal discharge, followed by lower abdominal pain (22.8%), vaginal itching (17.5%), fever (28.1%), and vaginal odour (3.5%), while 5.2% reported a history of previous pre-term delivery. A small proportion reported practising vaginal douching during pregnancy (3.5%), and 58.3% indicated recent antibiotic use. Distribution by gestational age showed that 5%, 28%, and 67% were in the first, second, and third trimesters, respectively.

Trichomonas vaginalis was detected in 33.3% of the samples. Analysis of demographic factors showed a statistically significant association between *T. vaginalis* infection and gestational age ($\chi^2 = 6.123$, $p = 0.047$), with the highest prevalence observed in the second trimester (56.5%). No statistically significant association was found between infection and age, level of education, marital status, occupation, type of marriage, or parity ($p > 0.05$) (Table: 1). Similarly, there was no significant association between infection and gynaecological or obstetric characteristics, including pre-term delivery, vaginal discharge, itching, odour, fever, lower abdominal pain, antibiotic therapy, or vaginal douching ($p > 0.05$) (Table 2).

Molecular Identification and BLAST Characterisation

Plate 1 displays the PCR products of the amplified TVKS gene from high vaginal swabs, confirming *T. vaginalis* infection. Molecular analysis identified five DNA extracts positive for the *T. vaginalis*-specific TVSK gene, verifying infection at the molecular level. Of the five samples that were microscopy-positive, four (80%) were confirmed by

PCR (Samples A1 – A5, Plate 1). Conversely, one out of five samples that tested negative via microscopy was positive by PCR (Samples B1 – B5, Plate 1), demonstrating improved sensitivity of molecular detection. Comparing microscopy to the gold standard PCR (NAAT) for diagnosing *T. vaginalis*, the test showed a sensitivity of 80% and a specificity of 80%. Accordingly, both the Positive Predictive Value (PPV) and the Negative Predictive Value (NPV) were calculated at 80% for this high-prevalence sample. The 80% sensitivity is considered suboptimal for public health screening, equating to a 20% false negative rate—that is, one in five genuinely infected individuals, including silent, asymptomatic carriers, are missed by microscopy. This failure to detect cases facilitates the spread of the infection and sustains its adverse public health effects, notably the risks of HIV transmission and preterm birth. The data underscores the importance of prioritising higher-sensitivity tests, such as NAAT, as recommended in the report.

Molecular sequencing and BLAST search results revealed that all DNA fragments amplified from the *Trichomonas vaginalis* isolates obtained in Ado-Ekiti showed significant homology with *T. vaginalis* G3 reference sequences. Alignment output indicated identity scores ranging from 86.18% to 94.35% against *T. vaginalis* DNA/RNA polymerase-family gene segments (Table 4.5). These values surpass the minimum threshold commonly accepted for reliable species-level identification in protozoan molecular diagnostics, confirming that the obtained isolates were genuine strains of *T. vaginalis*. The repeated matches to the G3 strain suggest that the genomic region targeted in this study aligns with a conserved hotspot within the *T. vaginalis* genome, especially within polymerase-associated coding regions.

Phylogenetic Analysis and Evolutionary Positioning

The phylogenetic tree (Figure 4.1) positioned all isolates within the same major clade as *T. vaginalis* G3, confirming shared ancestry and evolutionary proximity. However, the branch pattern showed sub-clustering, consistent with genetic heterogeneity among local isolates

Discussion

Socio-demographic Profile

The study population consisted of pregnant women aged 21–40 years (mean: 28.3 ± 10.0), predominantly within the economically active and reproductive age bracket. This age distribution aligns with reports indicating that *T. vaginalis* infection is most common among sexually active women aged 20–45 years (Francis et al., 2023; Muzny et al., 2020). Nearly all participants were married and in monogamous unions, reflecting typical cultural marital patterns in southwestern Nigeria. The high literacy level, where all respondents had at least secondary education, is noteworthy since prior studies have linked lower educational attainment with higher STI risk (Chikandiwa et al., 2022). However, education did not significantly influence infection status, supporting the claim that awareness does not automatically translate to protective behavioural practices (Omar et al., 2024).

Obstetric and Gynaecological Characteristics

Clinical symptoms reported included vaginal discharge (43.9%), lower abdominal pain (22.8%), itching (17.5%), and fever (28.1%). Although these symptoms are classical signs of trichomoniasis, *T. vaginalis* infection was not statistically associated with any of them. The lack of association supports evidence that more than 50% of infected women remain asymptomatic, making clinical diagnosis unreliable without laboratory confirmation (Rabiee et al., 2022; Sena & Bachmann, 2022). The high proportion of antibiotic use (58.3%) raises concerns regarding self-medication and non-targeted antimicrobial therapy, practices known to promote treatment failure, diagnostic masking, and antimicrobial resistance (Workowski et al., 2021).

Prevalence of T. vaginalis

The prevalence of 33.3% indicates a considerably high burden of trichomoniasis among pregnant women in the study location. This rate is higher than recent reports from Nigeria (14–26%) and other African nations, including Ghana (17.5%) and Ethiopia (12–21%) (Arinze et al., 2023; Apalata et al., 2022; Fenta et al., 2021). Differences in diagnostic performance, participant sexual behaviour, socio-cultural norms, and regional epidemiology may account for the variability. The reliance on **microscopy**, which is less sensitive than nucleic acid amplification tests, suggests that the actual prevalence may be underestimated (Muzny & Schwebke, 2020).

Socio-demographic Factors Influencing Infection

A statistically significant association was found between gestational age and infection ($\chi^2 = 6.123$, $p = 0.047$), with higher positivity rates in women in the second trimester. Hormonal shifts, altered immunity, and changes in vaginal flora during mid-pregnancy may favour protozoan colonisation (Kissinger et al., 2022). Conversely, no significant association was observed with age, education, marital status, occupation, type of marriage, or parity. This aligns with recent findings showing that *T. vaginalis* transmission is not necessarily dependent on socioeconomic or marital variables but rather on sexual exposure patterns, partner treatment compliance, and behavioural risk factors (Omar et al., 2024; Sena & Bachmann, 2022).

Clinical and Behavioural Correlates

Although symptomatic women exhibited infection, no symptoms were statistically correlated with *T. vaginalis*. Similar studies have emphasised that trichomoniasis should not be ruled out based solely on the absence of symptoms (Sena & Bachmann, 2022). Importantly, nearly all women who practised vaginal douching tested negative, although the sample size (n=8) was too small for meaningful inference. Douching is widely discouraged due to its disruptive effect on vaginal microbiota and established

association with STIs, pelvic inflammatory disease, and adverse pregnancy outcomes (Wessels et al., 2023). The lack of association between antibiotic use and infection suggests that many antibiotics accessed by participants may not target protozoal pathogens, further stressing the need for syndromic management review.

The overall findings reveal a high prevalence of trichomoniasis among antenatal attendees despite high literacy and predominantly stable marital status. The absence of a significant association with most sociodemographic, clinical, and behavioural variables, and the statistical association with gestational age, suggest that pregnancy-related physiological changes, rather than individual social characteristics, may play a more prominent role in infection susceptibility. Routine screening, rather than symptom-based diagnosis, is recommended to reduce maternal and neonatal risks.

Molecular Identification and BLAST Characterisation

The microscopy sensitivity of 80% reported here is higher than the 40–68% sensitivity often cited in existing literature, yet it still confirms the consensus that this method is suboptimal for public health screening compared to Nucleic Acid Amplification Tests (NAATs) (Rajaraman et al., 2014; U.S. Centres for Disease Control and Prevention, 2021). The findings support the current recommendation for prioritising NAATs, as their documented sensitivity ranges (95–100%) significantly reduce the high rate of missed infections (false negatives) associated with microscopy, which are crucial to address the transmission risks mentioned in the report (U.S. Centres for Disease Control and Prevention, 2021).

Molecular sequencing and BLAST search results revealed that all DNA fragments amplified from the *Trichomonas vaginalis* isolates obtained in Ado-Ekiti showed significant homology with *T. vaginalis* G3 reference sequences. Alignment output indicated identity scores ranging from 86.18% to 94.35% against *T. vaginalis* DNA/RNA polymerase-family gene segments. These values surpass the minimum threshold commonly accepted for reliable species-level identification in protozoan molecular diagnostics, confirming that the obtained isolates were genuine strains of *T. vaginalis*. The repeated matches to the G3 strain suggest that the genomic region targeted in this study aligns with a conserved hotspot within the *T. vaginalis* genome, especially within polymerase-associated coding regions.

The variation in percentage identity, however, indicates that the isolates are not genetically identical to existing GenBank reference sequences, reflecting intra-species polymorphism. Such diversity is well-documented in *T. vaginalis*, attributed to genome size expansion, large gene family repeats, horizontal gene acquisition, and recombination-linked mutation (Bradic et al., 2017; Carlton et al., 2007). The observed sequence divergence may therefore reflect micro-evolutionary events, host-driven selection, or drug-exposure history within the sampled population.

Phylogenetic Analysis and Evolutionary Positioning

The phylogenetic tree positioned all isolates within the same major clade as *T. vaginalis* G3, confirming shared ancestry and evolutionary proximity. However, the branch pattern showed sub-clustering, consistent with genetic heterogeneity among local isolates. Similar bifurcated phylogenetic structures have been reported globally, suggesting at least two dominant but recombining *T. vaginalis* population groups (Cornelius et al., 2012; Conrad et al., 2012). The sub-branching observed in the present study implies possible genotypic diversification, potentially linked to sexual network patterns, variable immune pressure, or differential exposure to antimicrobial agents.

The G3-related clustering further supports the hypothesis that *T. vaginalis* genotype circulation may be globally conserved, but local mutations accumulate over time, giving rise to region-specific genomic signatures. Given that *T. vaginalis* exhibits evidence of parasexual recombination and loss of linkage disequilibrium, such diversity is biologically plausible (Bradic *et al*., 2017).

Public Health and Clinical Significance

The genetic variability identified may have clinical and epidemiological implications. Previous studies suggest associations between certain *T. vaginalis* genotypes and metronidazole resistance, symptomatic infections, enhanced virulence, and viral coinfection, particularly with *T. vaginalis*-specific dsRNA viruses (TVV) (Snipes *et al*., 2000; Yadav *et al.,* 2018). Considering that *T. vaginalis* drug resistance is an emerging concern in Sub-Saharan Africa, the unique polymorphic sequences in this study may serve as early indicators of evolving phenotypic traits.

Conclusion and Recommendation:

This study demonstrates a high prevalence of *Trichomonas vaginalis* G3 among pregnant women in Ekiti State, indicating significant public health concerns due to its association with adverse maternal and neonatal outcomes, including preterm birth and low birth weight. The findings underscore the limitations of symptom-based diagnosis and conventional microscopy, emphasising the need for sensitive molecular screening methods. It is recommended that routine antenatal screening be implemented alongside public health education on sexual health and hygiene. Additionally, partner notification and treatment, coupled with monitoring for antimicrobial resistance, should be prioritised to reduce infection rates, prevent complications, and safeguard maternal and child health.

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Table 1: Relationship between *T. vaginalis* occurrence and socio-demographic characteristics of the study population

Characteristics	Total	*T. vaginalis* Negative	*T. vaginalis* Positive	χ^2	P value
Age (years)					
21-25	62	44 (71.0)	18 (29.0)	0.237	0.627
26-30	66	51 (77.3)	15 (22.7)		
31-35	70	48 (68.6)	22 (31.4)		
36-40	22	4(18.2)	18(81.8)		
Education					
None	0	0	0	0.055	0.815
Primary	0	0	0		
Secondary	40	26(65)	15(37.5)		
Tertiary	180	121(67.2)	59(32.8)		
Occupation					
Self-Employed	131	94(71.8)	37(28.2)	1.800	0.407
Employed	63	33(52.4)	30(47.6)		
Student	26	19(73.1)	7(26.9)		
Marital Status					
Single	4	0	4(100)	2.231	0.135
Married	216	146(67.6)	70(32.4)		
Occupation					
Self-Employed	131	94(71.8)	37(28.2)	1.800	0.407
Employed	63	33(52.4)	30(47.6)		
Student	26	19(73.1)	7(26.9)		
Type of Marriage					
Monogamy	213	143(67.1)	70(32.9)	0.244	0.621
Polygamy	7	4(57.1)	3(42.9)		
Parity					
0	96	77(80.2)	19(19.8)	6.199	0.185
1	85	46(54.1)	39(45.9)		
2	27	15(55.6)	12(44.4)		
3	8	8(100)	0		
s4	4	4(100)	0		
Gestation					
1st Trimester	11	11 (100)	0 (0)	6.123	0.047*
2nd Trimester	62	27 (42.5)	35 (56.5)		
3rd Trimester	147	108 (73.5)	39 (26.5)		

*Significant

Table 2: Relationship between *T. vaginalis* occurrence and gynaecological and obstetrics characteristics of the study population.

Characteristics	Total	*T. vaginalis* Negative	*T. vaginalis* Positive	χ^2	P value
Pre-term delivery					
No	209	144(68.9)	64(30.6)	0.008	0.930
Yes	11	8(72.7)	4(36.4)		
Vaginal Discharge					
No	123	77(62.6)	46(37.4)	0.575	0.448
Yes	97	70(72.2)	27(27.8)		
Vaginal itching					
No	182	124(68.1)	58(31.9)	0.095	0.758
Yes	39	24(61.5)	15(38.5)		
Offensive odour					
No	212	143(67.5)	69(32.5)	0.247	0.621
Yes	8	4(50)	4(50)		
Fever					
No	158	108(68.4)	50(31.6)	0.172	0.679
Yes	62	39(62.9)	23(37.1)		
Lower abdominal pain					
No	170	108(63.5)	62(36.5)	0.835	0.361
Yes	50	39(78)	11(22)		
Antibiotic treatment					
No	92	60(65.2)	32(34.8)	2.012	0.156
Yes	128	87(68)	41(32)		
Vaginal douching					
No	212	139(65.6)	73(34.4)	1.658	0.198
Yes	8	8(100)	0		
Smoking					
No	220	147(66.8)	73(33.2)		
Yes	0	0	0		

Table 3: Nearest Relatives of Sequenced DNA data of *Trichomonas vaginalis* isolates following BLAST

S/N	Nearest Relative	Name	Homology	Accession Number
1	*Trichomonas vaginalis* G3 DNA/RNA polymerases family (TVAGG3_1073780), partial mRNA	*Trichomonas vaginalis* G3	86.18%	XM_053888460.1
2	*Trichomonas vaginalis* G3 DNA/RNA polymerases family (TVAGG3_0383810), partial mRNA	*Trichomonas vaginalis* G3	92.96%	XM_051232160.1
3	*Trichomonas vaginalis* G3 DNA/RNA polymerases family (TVAGG3_0418090), partial mRNA	*Trichomonas vaginalis* G3	86.87%	XM_051233324.1
4	*Trichomonas vaginalis* G3 DNA/RNA polymerases family (TVAGG3_1068690), partial mRNA	*Trichomonas vaginalis* G3	89.90%	XM_051256295.1
5	*Trichomonas vaginalis* G3 DNA/RNA polymerases family (TVAGG3_1073780), partial mRNA	*Trichomonas vaginalis* G3	94.35%	XM_053888460.1

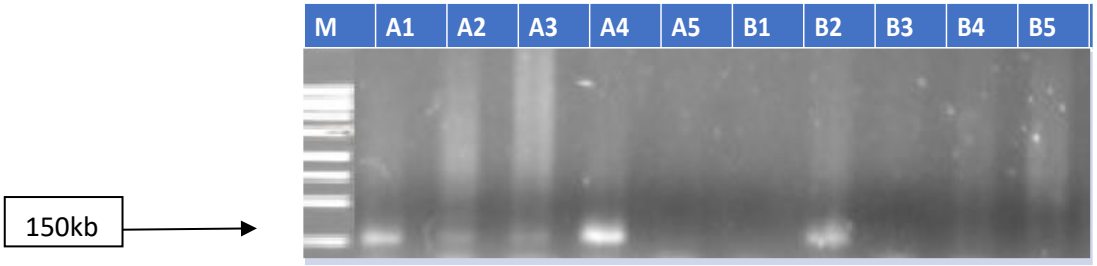

Plate 1: PCR product of the amplified TVKS gene of *Trichomonas vaginalis* from high vaginal swabs.
Key: M – Molecular standard marker; A1-A4 and B2 positive samples for *T. vaginalis*

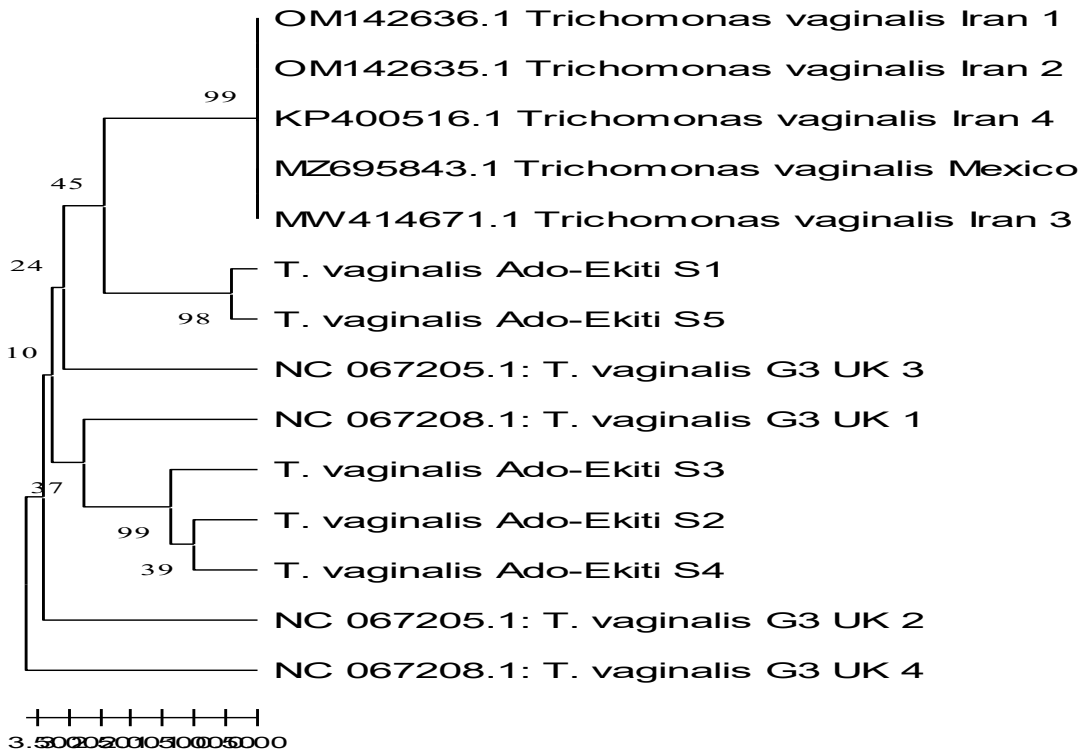

Figure 1: A phylogenetic comparison of *T. vaginalis* isolated from Ado-Ekiti with NCBI data entries

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