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Molecular detection of *Trichomonas Vaginalis* using 5.8S rRNA gene in reproductive-age women in Erbil city

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Abstract

Background and objective: Trichomoniasis is a parasitice infection that impacts the human reproductive and urinary systems, constituting a significant non-viral sexually transmitted infection globally. The study evaluated the 5.8S rRNA gene as a molecular target for diagnosing *T. vaginalis* infection among reproductive-age women in Erbil city.

Methods: Vaginal swabs and urine samples from 800 women were examined microscopically and by PCR targeting a 368 bp fragment of the 5.8S rRNA gene.

Results: Thirteen samples (1.63%) tested positive microscopically and were all confirmed by PCR, demonstrating 100% sensitivity and specificity.

All patients with positive microscopic identification were confirmed using the amplificati on of a 368bpfragment of 5.8S rRNA gene, acquisition of the accession number from the gene bank (NCBI) under accession numbers (PV935463, PX147457, PX147458, PX147459, PX147460, PX147461, PX147462, PX147463, PX147464, PX147465, PX147466, PX147467, PX218319).

Conclusion: Molecular detection targeting the 5.8S rRNA gene offers a highly sensitive and specific diagnostic tool for *Trichomonas vaginalis*, supporting its use alongside microscopy to improve detection accuracy in clinical settings.

Keywords: *Trichomonas vaginalis*, 5.8S rRNA gene, NCBI, Reproductive age, Erbil.

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Introduction

Trichomonas vaginalis is a flagellated protozoan parasite responsible for trichomoniasis, the most prevalent non-viral sexually transmitted infection worldwide(1). Humans are the only identified host, with transmission primarily occurring through sexual contact Importantly, approximately 50% of infected individuals remain asymptomatic, which facilitates silent transmission and poses significant challenges for effective diagnosis and disease control(2, 3). In symptomatic individuals, *T. vaginalis* infection presents with a range of clinical manifestations, including vaginal discharge, pruritus, dysuria, dyspareunia, and lower abdominal pain. Characteristic clinical findings may include a yellow-green frothy discharge and a "strawberry cervix" (4). In addition, *T. vaginalis* infection has been identified as a risk factor for the persistence of human papillomavirus infection and the development of cervical neoplasia (5). In men, the infection most commonly manifests as urethritis or prostatitis, although many cases remain asymptomatic (6). The global prevalence of the parasite varies considerably, with estimates between 2% and over 50%, driven by factors like geography, gender, and demographic characteristics. In high-risk populations, prevalence rates can rise above 10% (7). The public health importance of *T. vaginalis* extends beyond its high prevalence. Chronic infection has been associated with adverse reproductive outcomes, including preterm birth, low birth weight, and infertility. Moreover, trichomoniasis increases susceptibility to HIV acquisition and transmission, while effective treatment results in a substantial reduction in genital HIV-1 viral load (8, 9). Community awareness of infertility risk factors, including untreated reproductive tract infections, has been reported to be only moderate in the Kurdistan Region, highlighting the importance of accurate diagnosis and patient education (28). Furthermore, accurate and sensitive detection is essential, as conventional approaches like wet mount microscopy frequently overlook asymptomatic patients. Molecular diagnostics, encompassing nucleic acid amplification assays, exhibit enhanced sensitivity and specificity, hence facilitating improved detection in clinical and screening conditions (10-12). Despite advances in diagnostic techniques, *T. vaginalis* infection remains inadequately detected and reported in many settings. This underdiagnosis is attributed to factors such as limited awareness among healthcare providers, the lack of routine screening programs, and the asymptomatic nature of many infections. Addressing these challenges requires improved surveillance systems, strengthened public health initiatives, and enhanced diagnostic capacity (13). Accumulating evidence suggests

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a potential role of *T. vaginalis* in carcinogenesis. Systematic reviews and meta-analyses have demonstrated an association between *T. vaginalis* infection and an increased risk of cervical neoplasia, particularly among individuals co-infected with human papillomavirus (HPV). Additionally, several studies have explored the association between *T. vaginalis* infection and prostate cancer; however, the findings remain inconclusive (14). Diagnostic techniques for *T. vaginalis* infection have progressed throughout time. Conventional techniques, like saline wet mount microscopy, provide prompt results but exhibit restricted sensitivity. Molecular approaches, such as nucleic acid amplification tests (NAATs), provide enhanced sensitivity and specificity, enabling more precise identification, particularly in asymptomatic individuals (15). The aim of the study is to evaluate the 5.8S rRNA gene as a molecular target for diagnosing *T. vaginalis* infection among reproductive-age women in Erbil city.

Material and Methods

Sample collection:

This study included 800 women suspected of trichomoniasis who attended obstetrics and gynecology outpatient clinics and Pap smear units at Maternity Teaching and Daik Hospital, as well as six health centers, and private clinics in Erbil, Kurdistan Region, Iraq, from June 1st, 2024, to June 1st, 2025. Data were gathered from each patient using a questionnaire, following the acquisition of the participant's initial consent.

Method for Investigating Vaginal Secretions

Vaginal secretions from the posterior vaginal area were separately collected using two sterile cotton swabs. One of the swabs was utilized for wet preparation to detect motile organisms by microscopic analysis. The other swab was immediately placed into a sterile tube containing 1 ml of sterile Phosphate Saline buffer (1X) (PBS) and stored at -20°C for molecular analysis. Additionally, 20–50 ml of urine was collected separately from the same patients in sterile containers and subsequently centrifuged. Samples confirmed as positive by microscopic examination were further analyzed using molecular techniques.

DNA extraction and PCR amplification

In the molecuolar laboratory at college of science, the extraction of DNA was conducted in accordance with the manufacturer's instructions using Animal and Fungi DNA preparation-solution kit (Jena Bioscience, Germany). The DNA yield was measured for quantity and purity at 280/260 nm via Nanodrop (Thermo Scientific, USA). Subsequently, conventional PCR was performed targeting 5.8S rRNA gene for *Trichomonas vaginalis*, by using the primer TFR 1 (5' TGC TTC AGT TCA GCG

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GGT CTT CC 3') and TFR 2 (5' CGG TAG GTG AAC CTG CCG TTG G3')(16).The PCR amplification for the 5.8S rRNA gene comprised a total volume of 50 µl, including 25 µl of Go Taq Green Master Mix (Promega/ USA), 3 µl of genomic DNA, 1.5 µl of each primer, and 19 µl of nuclease-free water to complete the volume. PCR amplification was performed under the following conditions: The first 4 minutes are for Taq-polymerase enzyme activation in which the dsDNA is also dissociated, followed by 38 cycles of denaturation at 94 °C for 35 seconds, annealing at 62 °C for 30 seconds, and extension at 72 °C for 1 minute. A final extension step was carried out at 72 °C for 10 minutes. The PCR products were analyzed by electrophoresis on a 2% agarose gel, and DNA bands were visualized using a Safe dye. The presence and absence of genes were assessed using a UV trans illuminator (Syngene/ UK). The amplicon bands for 5.8S rRNA genes were visualized on the gel relative to the standard DNA ladder of 1kb range (Froggabio, / Canada).

PCR products of isolates for both direction (forward and reverse) primers were dispatched to Macrogen in South Korea for sequencing. All sequencing files were analyzed using MEGA11 and subsequently aligned with NCBI-BLAST.

Results

Out of 800 specimens, 13 (1.63%) tested positive for *T. vaginalis* infection upon microscopic analysis (Figure 1). These included 10 individuals (1.25%) identified via posterior vaginal fornix discharge analysis and 3 individuals (0.38%) identified through urine analysis.

Figure 1. Trichomonas vaginalis under microscope (400X)

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The 5.8S rRNA gene was targeted for the molecular detection of the parasite. All microscopically positive tested samples were further analyzed by PCR amplification of a 368bp fragment of the 5.8S rRNA gene (Figure 2). The molecular analysis yielded 100% sensitivity and specificity for the detection of *T. vaginalis*. The sequences obtained in this study were submitted to GenBank under the accession numbers PV935463, PX147457, PX147458, PX147459, PX147460, PX147461, PX147462, PX147463, PX147464, PX147465, PX147466, PX147467, and PX218319.

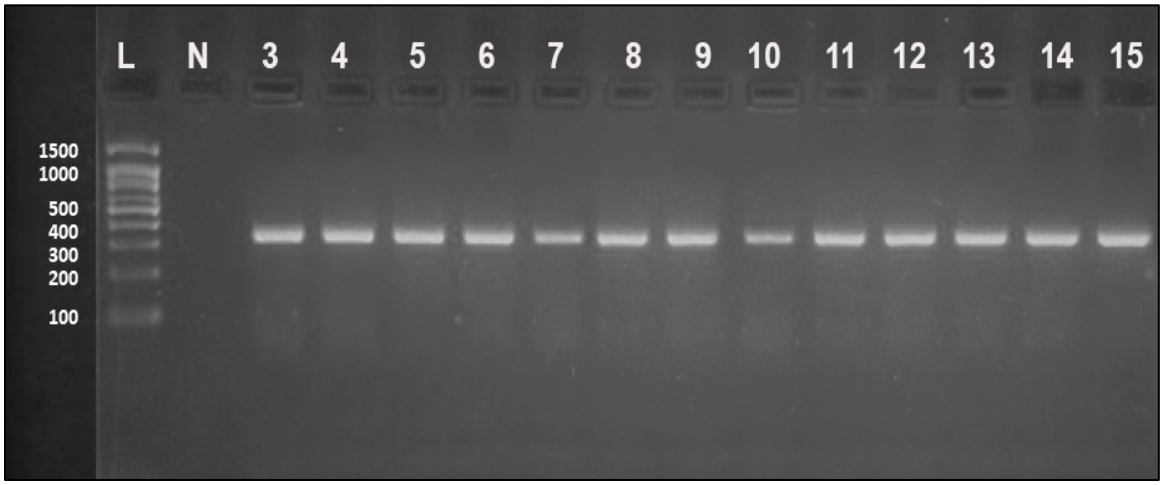

Figure 2. Agarose gel electrophoresis of PCR products targeting the 5.8S rRNA gene and internal transcribed spacer (ITS) regions of Trichomonas vaginalis (368 bp). Lane L: 100 bp DNA ladder; Lane N: nuclease-free water (negative control); Lanes 3–15: T. vaginalis–positive isolates.

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The Phylogenetic tree of Trichomonas vaginalis isolates (Figure 3) based on TFR region sequences generated by using Sanger sequencing. Chromatograms were examined and refined, forward and reverse reads were compiled to generate consensus sequences, and sequences were matched with reference sequences obtained from GenBank. The tree was generated with Maximum Likelihood with 1000 bootstrap replicates. Bootstrap values (%) are displayed at critical nodes; the scale bar represents substitutions per site.

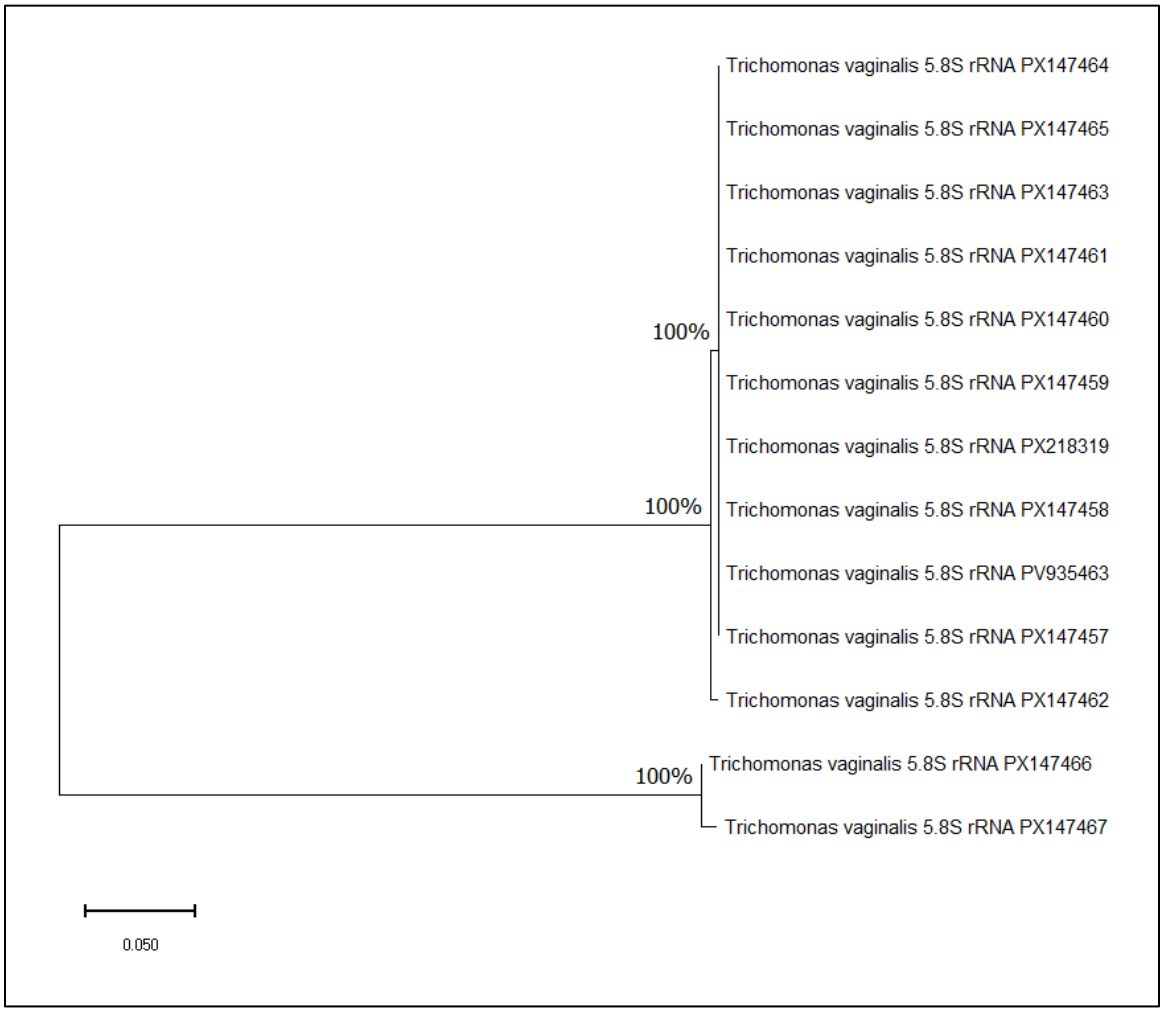

Figure 3. Phylogenetic tree constructed based on ITS1–5.8S rRNA–ITS2 sequences of *Trichomonas vaginalis* isolates amplified using primers TFR1 and TFR2, showing the relationships among representative *T. vaginalis* sequences. The numbers at the nodes indicate bootstrap support values (%) based on Maximum Likelihood analysis of 100 replicate datasets. The scale bar represents a phylogenetic distance of 0.050 substitutions per 100 nucleotidepositions.

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Discussion

Different microbiological organisms are associated with women's vaginal discharge, with trichomoniasis being the most common sexually transmitted disease (STD) worldwide. The worldwide prevalence of trichomoniasis is estimated to range from 5% to 74% (17, 18). A study done in Duhok city indicated a prevalence of 3.2% (24 out of 400) among women with vaginal infections (19). Also in Wasit, another researcher found 4% (4/100) prevalence with trichomoniasis (20). An additional study done in Kirkuk found the presence of *T. vaginalis* was identified in 2.8% of samples (21), these studies are relatively comparable to our study. Vaginal infections in the Kurdistan Region are not limited to trichomoniasis; a study on pregnant women in Sulaymaniyah and Erbil cities reported vaginal candidiasis in about a third of participants, underscoring the broader burden of reproductive tract infections among women in this region (29). Conversely, other researchers in Brazil indicated that 9% of patients tested positive for *T. vaginalis* infection via direct examination (22). Furthermore, in Turkey, the prevalence of the parasite among women reporting with vaginal discharge was assessed using wet-mount and Giemsa staining techniques, revealing an infection rate of 9% (23). he identification of infection is a crucial component in influencing the incidence rate of trichomoniasis, utilizing several methods, from clinical diagnosis to laboratory procedures. Microscopic analysis of a vaginal wet mount is a crucial technique for identifying *T. vaginalis* infection (24). The sensitivity range of wet mount microscopic examination is reported to be 44-68%. The wet mount technique is rapid and cost-effective; nevertheless, the accuracy of this diagnostic test is significantly influenced by the technician's expertise and familiarity with the microscope (24, 25). Liquid-based preparations have demonstrated efficacy in diagnosing *T. vaginalis*. Women identified as carriers of the organism are advised to undertake confirmatory testing. Recent studies indicate that molecular techniques, such as PCR, exhibit outstanding specificity and sensitivity, providing more reliable estimations (26, 27). In our investigation, 13 samples were identified as positive for infection by direct microscopic examination and subsequently analyzed using PCR. Molecular amplification indicated that all 13 samples tested positive, using the 5.8S rRNA gene as the target for parasite identification.

The phylogenetic tree of *Trichomonas vaginalis*, based on the 5.8S rRNA gene, demonstrates considerable genetic similarity among isolates, as seen by short branch lengths and high bootstrap values (100%) validating most clades. This indicates that the 5.8S rRNA region

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is significantly conserved in *T. vaginalis*, providing a reliable identifier for species identification.

Conclusion

The research showed that *Trichomonas vaginalis* infection was identified in 13 (1.63%) of 800 analyzed samples with direct microscopy, and all positive samples were subsequently confirmed by PCR with complete concordance. This finding indicates that PCR can verify *T. vaginalis* infection in microscopy-positive samples, supporting its use as a supplementary tool to conventional microscopic examination for accurate diagnosis.

Competing interest

The authors declare that they have no competing interests.

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