Available online at www.garmian.edu.krd

Passer Journal of Basic and Applied Sciences

Passer (Issue 1) (2026) 472-477

Original Article

Molecular Characterization of *Trichomonas vaginalis* in Reproductive-Age Women in Erbil City

Hawri Hawar Mohammed*[1], Zuber Ismail Hassan[1], Hero Mohammad Ismael[2]

[1]*Department of Medical Laboratory technology, Erbil Technical Health and Medical College, Erbil Polytechnic University, Erbil, Kurdistan Reigen, Iraq.*
[2]*Department of Biology, College of Science, Salahaddin University, Erbil, Kurdistan Reigen, Iraq.*

Received 23 October 2025; revised 20 January 2026;
accepted 02 February 2026; available online 17 February 2026

DOI: 10.24271/PSR.2026.554994.2416

ABSTRACT

Background: *Trichomonas vaginalis* is the etiological agent of trichomoniasis, one of the most prevalent non-viral sexually transmitted diseases globally, particularly among immunocompromised women, such as those who are pregnant.
Objectives: This study aims to molecularly characterise, perform phylogenetic analysis, and investigate some sociodemographic factors associated with trichomoniasis in women at reproductive age.
Materials and methods: Included 800 vaginal swabs and urine samples were collected from women who attended Maternity Hospital, different centers and private clinics in Erbil City. The patients were grouped according to their ages, residency and educational level factors. Samples were examined by microscopic examination and nucleic acid identification of the *AP65-1* gene and the surface immunogen (*p270*) gene.
Results: Among samples collected, 13 (1.63%) were positive for *T. vaginalis* infection following microscopic examination. The vaginal swab test showed that 10 individuals (1.25%) identified through posterior vaginal fornix secretion examination and 3 individuals (0.38%) identified through urine examination. The molecular analysis for the *AP65-1* gene identified 11 positive cases, in comparison to the conventional approaches, while molecular screening for the surface immunogen (*p270*) gene showed a lower, only 4 positive cases, acquisition of the accession number from the gene bank (NCBI) for all samples in both genes separately.
Conclusion: The examination of the data gathered during this survey shows a statistically significant correlation between trichomoniasis and education level. AP65-1 gene has a higher degree of sensitivity compared with conventional approaches, while the surface immunogen (*p270*) gene has less.

Keywords: Ap65-1 gene, surface immunogen (P270) gene, NCBI, Trichomonas vaginalis, Trichomoniasis.

1 Introduction

Trichomonas vaginalis is a highly mobile (capable of movement), aerotolerant eukaryotic which inhabits urogenital tract of human [1]. Trichomoniasis is majority prevalent non-viral sexually transmitted infection globally, especially among groups exhibiting high-risk behaviors, including inadequate sexual hygiene or several sexual partners [2]. Centres for disease Control and Prevention (CDC) has categorised an ignored parasitic illness and advises for specific actions to prevent the sickness [3,1].

* Corresponding author
E-mail address hawri.mohammed@epu.edu.iq (Instructor).
Peer-reviewed under the responsibility of the University of Garmian.

The incidence rates approach those of Chlamydia, gonorrhea, an d syphilis infections [4,5]. Certain persons with vaginal trichomoniasis exhibit no obvious clinical signs, leaving asymptomatic carriers potential "sources of infection" and thus increasing the general health burden. Thus, the early identification and efficient management of vaginal trichomoniasis infection have emerged as significant public health issues globally [6]. Post-infection, patients may exhibit common symptoms including heightened vaginal discharge, smell, pruritus, burning sensations, and genital erythema. In more acute instances, symptoms may escalate to abdominal pain, increased urinary frequency, and dysuria [7].

This parasite transmits directly between hosts by sexual intercourse and indirectly through many means, using contaminated lavatories, undergarments, and towels belonging to

the infected individual. Additionally, transferred during physicians' examining patients' sexual organs through the use of unclean medical instruments, which is regarded as a significant source of transmission [8,9]. Patients infected with *T. vaginalis* were one-half more probable to develop Human Immunodeficiency Virus than those with infection-free with the parasite [10]. The vaginal pH, normally around 4.5, frequently increases to above 5 or 6 during a *T. vaginalis* infection [11]. Women are at an elevated risk of acquiring infections compared to men [12]. In men, the condition frequently manifests as "clinically asymptomatic," but dysuria, urethritis, epididymitis, and prostatitis are recognized symptoms [13]. A potential risk factor for developing cervical cancer may increase triple [14,15]. Recent investigations have demonstrated that *T. vaginalis* is implicated in the aetiology of cancer in cervical and prostate in both women and men respectively [16,4]. Variation in reported prevalence of trichomoniasis may result from the utilization of diverse diagnostic methods and the characteristics of the researched participants [17].

2 Material and Methods

2.1 Sample collection

The descriptive cross-sectional study included 800 women suspected of trichomoniasis, who visited Maternity Hospital, various centers (Mala Afandy, Dr. Mohammed Bajalan, Dr. Najdi Haydar, Bastora, Mala Omer and Sarkarez), and some private clinics in Erbil City, Kurdistan Region, Iraq., from June 2024, to June 2025. Data were gathered from each participant by a questionnaire, following the acquisition of the participant's initial consent.

2.2 Method for Investigating Vaginal Secretions.

The vaginal secretion from the posterior vaginal region was collected individually using two sterile cotton swabs. A single swab was utilized for wet preparation to detect the existence of motile organisms using microscopic analysis. While another swab was promptly injected into a sterile tube include 1 ml of sterile Phosphate-Buffered Saline (PBS) and stored at -20 ˚C for molecular analysis. Furthermore, twenty to fifty ml urine was gathered individually into sterile container from the same patient. The samples underwent centrifugation at 3000 revolutions per minute for 5 minutes. The isolates confirmed as positive through microscopy underwent additional analysis via a molecular technique.

2.3 Molecular Analysis for *AP65-1* gene with polymerase chain reaction (PCR).

The DNA extraction was performed following the manufacturer's guidelines, utilizing the Animal and Fungi DNA preparation solution kit (Jena Bioscience, Germany). The DNA yield was quantified using a Nanodrop (Thermo Scientific, USA). Subsequently, traditional PCR was performed targeting the AP65-1 gene of *Trichomonas vaginalis*, utilizing the primer (F:5' ATGTTCGATCACCGTGGCAT3' and R:5' TTGACTTGTTCGGCTGGGAA3') [18]. The polymerase chain reaction for the *AP65-1* gene comprised a total volume of 50 µl, including 25 µl of Gotaq Green Master Mix (Promega/ USA), 3 µl of genomic DNA, 1.5 µl of each primer, and 19 µl of nuclease-free water to finalize the volume. The PCR was conducted under the specified conditions: initial denaturation at 95°C for 5 minutes. The amplification comprises 35 cycles: denaturation at 94°C for 35 seconds, annealing at 62°C for 1 minute, and extension at 72°C for 1 minute, concluding with a final extension at 72°C for 8 minutes. The PCR amplicons were subsequently examined using 2% agarose gel electrophoresis, with the DNA stained with Safe dye. The presence and absence of genes were assessed using a UV trans illuminator (Syngene/ UK), and the polymerase chain reaction product bands for *AP65-1* genes were visualized on the gel relative to the standard DNA ladder/1kb (Froggabio/ Canada).

2.4 Molecular Analysis for surface immunogen (*p270*) gene with polymerase chain reaction (PCR)

The p270 gene master mix (Promega/ USA) was produced in a total amount of 50 µl. 25 µl of Gotaq Green Master Mix, 3 µl of genomic DNA, and 1.5 µl for each newly designed primer, the Forward primer: '5-GCTGCCAGTAAAAGCCAGTG-3' and Reverse Primer: '5-AGAAACACTTTGGCCGGCTT-3' (This primer is our own work that design specifically for this study). Subsequently, the volume was finalized with 19µl of DNase and RNase-free water. The program cycle begins with an initial denaturation at 94°C for 5 minutes. The amplification consists of 35 cycles: denaturation at 94°C for 35 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 50 seconds, subsequent to a final extension at 72°C for 8 minutes. All isolates were examined for the *P270* gene utilizing the PCR approach by amplifying the gene-specific targeted sequence in a thermocycler (Techne/ UK). Polymerase chain reaction amplicons product were subsequently studied by 2% agarose gel electrophoresis, with DNA staining performed using Safe dye (Bioland/ USA). The existence and nonexistence of the surface immunogen (*P270*) was assessed by UV trans illuminator (Syngene/ UK), and the PCR band product for both genes (774 bp) was recognized upon the gel in comparison to the standard DNA ladder/1 kb (Froggabio/ Canada).

Statistical analysis

The Pearson chi-square test used to assess relationship among several sociodemographic in relating with the disease, use SPSS version 27 software. Statistical significance was defined as a p-value less than (0.05).

3 Result

A total of 800 women were examined, among these specimens, 13(1.63%) were positive for *T. vaginalis* infection following microscopic examination (Figure 1). These comprised 10 individuals (1.25%) identified through posterior vaginal fornix

secretion examination and 3 individuals (0.38%) identified through urine examination (Table 1).

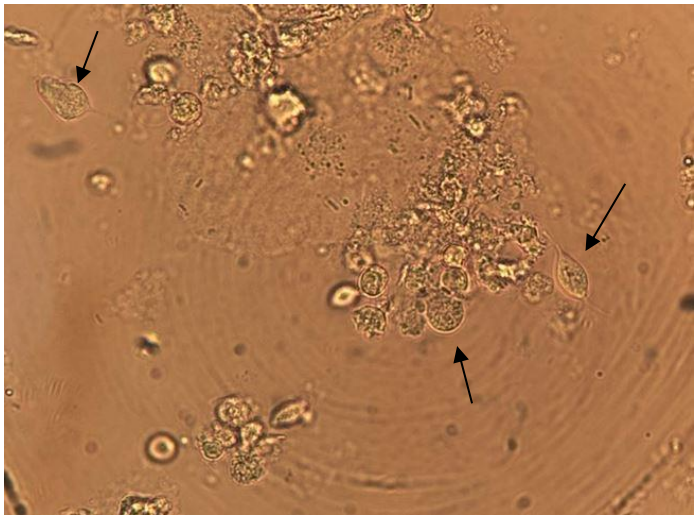

Figure 1: *Trichomonas vaginalis* under microscope (400X).

Table 1: Detection of *Trichomonas vaginalis* according to the samples.

Type of samples	Number examined	Number of Negative	Number of Positive	%
Posterior vaginal swab	800	790	10	1.25
Urine sample	800	797	3	0.38
Total	800	787	13	1.63

The affected women were aged between 16 and 50 years, with a higher positivity rate of 3% observed in age group 16-25 (Table 2). While for residency factor, 324 females were from urban and 476 from rural areas; 13 cases were positive for *T. vaginalis* (5 in urban and 8 in rural areas). The total rate of infection was 1.63% (1.54% in urban and 1.68% in rural areas) (Table 3).

Table 2: Prevalence of *T. vaginalis* according to the participants age group.

Age (years)	No. examined	No. of Positive	No. of Negative	%	P. value
16-25	133	4	129	3.0	0.10
26-35	343	8	335	2.33	
36-45	269	1	268	0.37	
46>	55	0	55	0	
Total	800	13	787	1.63	

Furthermore, regarding the educational status of the participants and the higher infection rate of trichomoniasis were almost among women who had a college education and school level, 4.76% and 4.21% respectively, compared to 1.06% with an illiterate education (Table 4).

Table 3: Prevalence of *T. vaginalis* according to the Residency of the participants.

Residency	No. examined	No. of Positive	No. of Negative	%	P. value
Urban	324	5	319	1.54	0.88
Rural	476	8	468	1.68	
Total	800	13	787	1.63	

Table 4: Prevalence of *T. vaginalis* according to the educational status of the participants.

Education level	No. examined	No. of Positive	No. of Negative	%	P. value
Illiterate	663	7	656	1.06	0.01
School	95	4	91	4.21	
University	42	2	40	4.76	
Total	800	13	787	1.63	

Statistical analysis of the data in this study between trichomoniasis and the specified parameters (including age, residency, and educational level). Significant association was detected between trichomoniasis and education level; however, no significant relationship was noted with residency and age groups.

3.1 *AP65-1* gene

The *AP65-1* gene (329bp) was examined for two objectives. The primary objective to confirm the existence of *T. vaginalis*, while the secondary goal to assess the detection of crucial virulence factors that facilitate the protozoan's adhesion to host cell surfaces A highly specialized process regulated by adhesion proteins, constituting an early and essential phase in colonization. The proteins of *AP65* may be secreted by the trophozoites of parasite and attach to the surfaces of both trophozoites stage and cells of the host [18]. Nucleic acid detection of the *AP65-1* gene by amplifying 329 bp fragment of the gene using the PCR method (Figure 2).

The molecular evaluating for the *AP65-1* gene, which encodes the exterior attachment protein, shown a heightened sensitivity level of 100 at 84.61 level of specificity, surpassing other conventional approaches. Then sequences acquired in this investigation were submitted to GenBank with the accession codes (PV942086, PX138256, PX399468, PX399469, PX399470, PX399471, PX399472, PX399473, PX399474, PX399475, PX399476) for the gene.

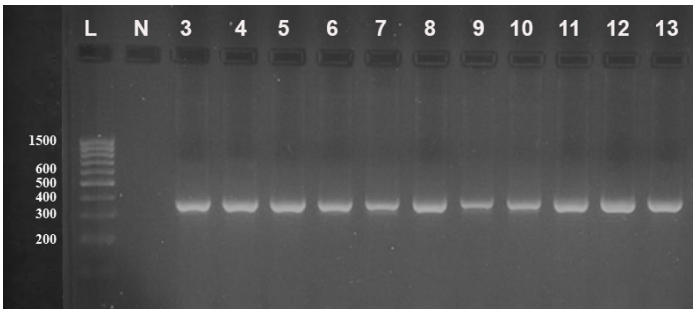

Figure 2: Agarose Gel Electrophoresis for surface adhesion gene. Lane L: Ladder 100bp, Lane N: nuclease free water as negative control, Lane 3-13: *AP65-1* gene (329bp).

The phylogenetic tree based on the *AP65-1* adhesion gene sequences of *Trichomonas vaginalis* isolates revealed two primary clusters with 100% bootstrap support, indicating strong reliability of the classification and then compared with the nucleotide sequences retrieved from GeneBank (Figure 3).

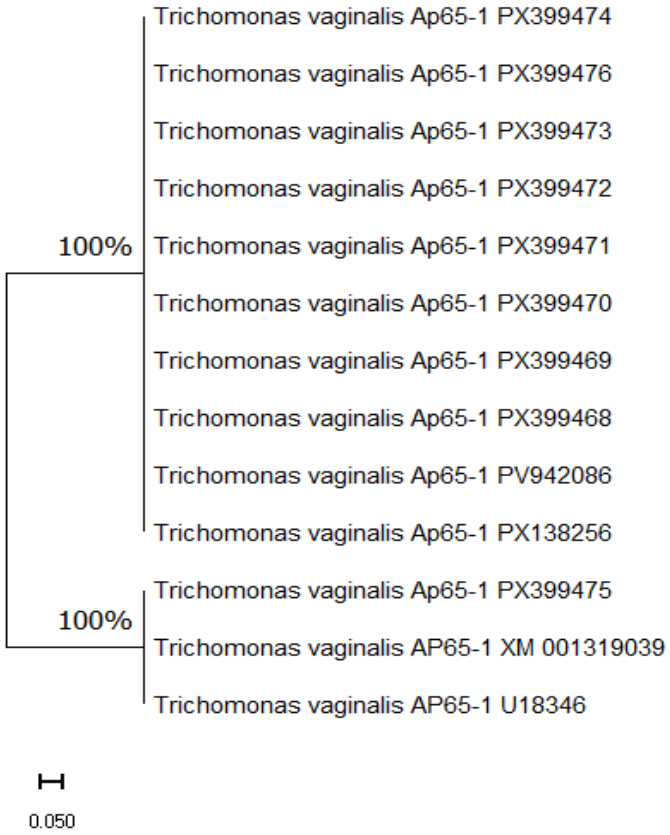

Figure 3: Phylogenetic tree for *Trichomonas vaginalis AP65-1* adhesion (*AP65-1*): showing relationship between representative sequences, XM_001319039 and U18346 nucleotide sequences of *T. vaginalis* retrieved from GenBank. The numbers at the nodes indicate the level of bootstrap support (%) based on Maximum Likelihood analysis of 100 re-sample datasets. The scale bar indicates the phylogenetic distance corresponding to 0.01 changes per 100 bases.

3.2 Surface immunogen (*p270*) gene

Out of 13 positive samples, the result of molecular screening for surface immunogen (*p270*) gene showed less sensitivity level of 100 at 30.76 in compare with other routine methods, then sequences acquired in this investigation were submitted to GenBank with the accession codes (PX259956, PX259957, PX259958, PX259959) for the gene. Nucleic acid discovery amplifying the 774 bp segment of the surface immunogen (*p270*) gene (Figure 4).

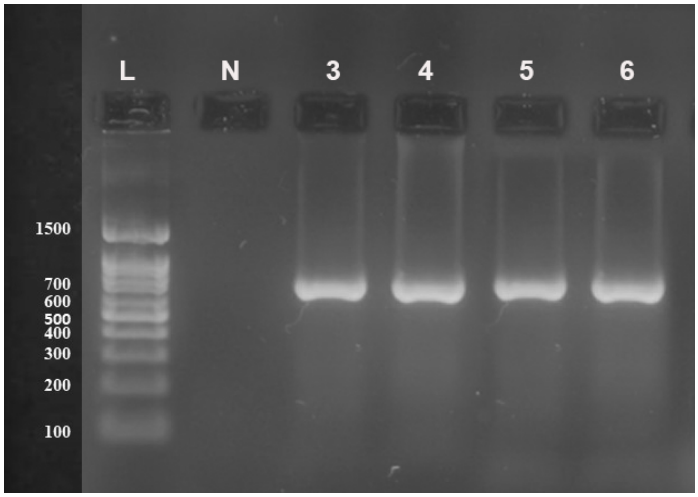

Figure 4: Agarose Gel Electrophoresis for surface immunogen. Lane L: Ladder 100bp, Lane N: Negative control, Lane 3-6: *P270* gene (774bp).

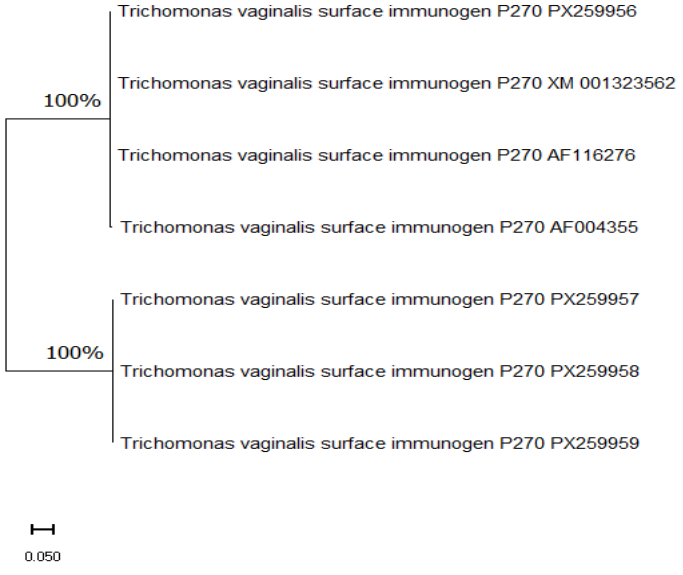

Figure 5: Phylogenetic tree based on Surface Immunogen *p270* gene sequences isolates showing relationship between representative *Trichomonas vaginalis* sequences, XM_001323562, AF116276 and AF004355 nucleotide sequences of *T. vaginalis* retrieved from GenBank. The numbers at the nods indicate the level of bootstrap support (%) base on Maximum Likelihood analysis of 100 resample datasets. The scale bar indicates the phylogenetic distance corresponding to 0.050 changes per 100 bases, followed by accession numbers of the sample.

The phylogenetic study of the *P270* gene from *Trichomonas vaginalis* isolates, comprising sequences PX259956, PX259957, PX259958, and PX259959, then (XM_001323562, AF116276 and AF004355) nucleotide sequences retrieved from GeneBank which is shown in (Figure 5).

4 Discussion

This study was conducted in Erbil city and examined the diagnosis of trichomoniasis in women of reproductive age. The current investigation identified the lowest incidence of *T. vaginalis* infections in urine samples, with a prevalence of 3 (0.38%). The subsequent stage involved employing a wet mount preparation, the predominant method of testing nowadays in laboratories [19], 10 (1.25%) tested positive for trichomoniasis infection. Nonetheless, this procedure is inaccurate and has limited sensitivity relative to alternative methods, causing the parasite to rapidly lose motility if subjected to any delay prior to this test [20]. Consequently, alternative methodologies should be explored that can yield more precise outcomes at a reduced cost and duration [21].

The study observed some sociodemographic characteristics connected to the presence of disease, including age, residency, and education level. A statistically significant correlation was found between trichomoniasis and education level; however, no correlation was observed between residency and age group. Incidence of the parasite infection among women at reproductive age may correlate with elevated sexual activity, which enhances reproductive hormone levels and reduces with advancing age [22]. Moreover, numerous factors contribute to variations in infection rates across different age demographics, including the secretion of estrogen and progesterone hormones that regulate vaginal pH during reproductive years, as well as the impact of abortion, frequency of pregnancies, immunocompromised states following menstruation, and vaginal pH levels [23].

Socioeconomic variables, including low educational attainment and limited economic income, along with heightened sexual desire, are correlated with an increased incidence of trichomoniasis [24]. In the present investigation, it was shown that a low level of education appeared to protect against trichomoniasis in urban areas. The reason for this may revert to limited health education and awareness lower literacy and educational attainment, socio-economic disadvantages, reduced access to healthcare services, poor hygiene and cultural practices [8].

Molecular techniques also used to detect parasites in research specimens. The procedures are new and have been devised for the detection of illnesses such as trichomoniasis [25]. In Polymerase Chain Reaction, a single molecule of DNA can be amplified to produce a million copies from one target cell [26].

The two genes selected for this objective are the Ap65-1 gene, amplified to generate a 329 bp fragment, and the surface immunogen (*p270*) gene, amplified to produce a 774 bp segment. The molecular evaluation for the *AP65-1* gene, which encodes the exterior attachment protein, shown a heightened sensitivity level, surpassing other conventional approaches. The observed clustering with 100% bootstrap values signifies that the *AP65-1* adhesion gene is extensively conserved throughout the analyzed *T. vaginalis* isolates, consistent with its recognized role as a crucial adhesion factor in the parasite's attachment to the vaginal epithelium (Figure 3).

While the molecular result for the surface immunogen (*p270*) gene shows less sensitivity level when compare with other routine methods. P270 gene of *Trichomonas vaginalis* encodes a significant surface protein (~270 kDa) that demonstrates phenotypic diversity, affecting the parasite's interaction with the host immune system. The fluctuation in P270 expression enables *T. vaginalis* to adjust to various host conditions and avoid immune recognition. The surface expression of P270 may augment interactions with host cells, whereas cytoplasmic localization may facilitate the parasite's evasion of immune monitoring. This phenotypic plasticity is essential for the parasite's survival and pathogenicity [27].

Over the past two decades, the prevalence of sexually transmitted diseases (STIs) has significantly risen, constituting a major public health issue in developing nations. Numerous investigations of disease incidence have been undertaken in Iraq. In Erbil, an investigation conducted by [7] found that among 440 women, 14 (3.18%) tested positive for trichomoniasis using culture techniques, and 12 (2.73%) tested positive via direct wet mount. Additionally, [28] identified five (0.39%) positive cases out of 1296 women referred to various hospitals and health centers in Erbil city for suspected Trichomonas infection, confirmed through direct microscopic examination and various vaginal smear methods. Additionally, researcher in Kirkuk [29] found the prevalence of pathogenic agents in urine samples was lower to that found in vaginal swabs. Although epidemiological data are lacking in certain places, there is surprisingly limited knowledge regarding the molecular techniques and sequencing of *Trichomonas vaginalis* in Erbil. Moreover, acknowledgement of disease distribution in certain regions of this city remains insufficient.

Conclusions and Recommendation

Trichomonas vaginalis rate occur low in Erbil (13/800; 1.63%), and women who are aged between 16-25 years old (3.0%) were highly affected by the disease, associated significantly with education level. While no significant correlation with the age and residency factors. The result of molecular for *AP65-1* gene that encodes for the surface attachment protein presented great level sensitivity in conjunction with others routine ways, while molecular screening for surface immunogen (*p270*) gene showed less. Analyze *AP65-1* sequences from different geographic isolates to identify genetic diversity. Further research is

recommended to investigate the relationship between the AP65-1 gene and iron levels; does its expression increase or decrease with varying iron concentrations?

References

[1] Herath, S., Balendran, T., Herath, A., Iddawela, D. & Wickramasinghe, S. Comparison of diagnostic methods and analysis of socio-demographic factors associated with *Trichomonas vaginalis* infection in Sri Lanka. *PLOS ONE* **16**(10), e0258556 (2021).

[2] Arbabi, M., Delavari, M., Fakhrieh-Kashan, Z., Hooshyar, H. Review of *Trichomonas vaginalis* in Iran, based on epidemiological situation. J. Reprod. Infertil. **19**, 82, (2018).

[3] Secor, W.E., Meites, E., Starr, M.C., Workowski, K.A. Neglected parasitic infections in the United States: Trichomoniasis. *Am. J. Trop. Med. Hyg.* **90**, 800, (2014).

[4] Galego, G.B., Tasca, T. Infinity war: *Trichomonas vaginalis* and interactions with host immune response. *Microb. Cell* **10**, 103, (2023).

[5] Aziz, A.A., Abdulaziz, S.M. Prevalence and clinical characteristics of pediatric rhinovirus infection during winter in Erbil City, Iraq. *Passer J. Basic Appl. Sci.* **7**, 1012–1020, (2025).

[6] Kim, T.G., Young, M.R., Goggins, E.R., Williams, R.E., HogenEsch, E., Workowski, K.A., et al. *Trichomonas vaginalis* in pregnancy: patterns and predictors of testing, infection, and treatment. *Obstet. Gynecol.* **135**, 1136–1144, (2020).

[7] Lv, G., Cao, X., Zheng, C. Clinical characteristics of vaginal trichomoniasis infection and metronidazole resistance in vaginitis patients. *Infect. Drug Resist.* 1161–1169, (2025).

[8] Nouraddin, A.S., Alsakee, H.M. Prevalence of *Trichomonas vaginalis* infection among women in Erbil governorate, Northern Iraq: An epidemiological approach. *Eur. Sci. J.* **11**, (2015).

[9] Murad, M.A., Ibrahim, S.A., Mahmood, A.Y. Distribution and risk factors of *Trichomonas vaginalis* infection with relevance to interleukin profile among population in Duhok city, Iraq. *Clin. Epidemiol. Glob. Health* **30**, 101784, (2024).

[10] Masha, S.C., Cools, P., Sanders, E.J., Vaneechoutte, M., Crucitti, T. *Trichomonas vaginalis* and HIV infection acquisition: A systematic review and meta-analysis. *Sex. Transm. Infect.* **95**, 36–42, (2019).

[11] Lin, Y.-P., Chen, W.-C., Cheng, C.-M., Shen, C.-J. Vaginal pH value for clinical diagnosis and treatment of common vaginitis. *Diagnostics* **11**, 1996, (2021).

[12] Daugherty, M., Glynn, K., Byler, T. Prevalence of *Trichomonas vaginalis* infection among US males, 2013–2016. *Clin. Infect. Dis.* **68**, 460–465, (2019).

[13] Doxtader, E.E., Elsheikh, T.M. Diagnosis of trichomoniasis in men by urine cytology. *Cancer Cytopathol* **125**, 55–59, (2017).

[14] Belfort, I.K.P., Cunha, A.P.A., Mendes, F.P.B., Galvão-Moreira, L.V., Lemos, R.G., de Lima Costa, L.H., et al. *Trichomonas vaginalis* as a risk factor for human papillomavirus: A study with women undergoing cervical cancer screening in a northeast region of Brazil. *BMC Womens Health* **21**, 174, (2021).

[15] Mahmud, A.M., Shawis, T.N., Hamid, S.F., Raheem, S.G., Karim, Y.A. Uncomplicated cystitis in female patients in Erbil City: What is the best antibiotic according to culture and sensitivity? *Passer J. Basic Appl. Sci.* **7**, 921–928, (2025).

[16] Zhang, Z., Li, D., Li, Y., Zhang, R., Xie, X., Yao, Y., et al. The correlation between *Trichomonas vaginalis* infection and reproductive system cancer: A systematic review and meta-analysis. *Infect. Agents Cancer* **18**, 15, (2023).

[17] Garcia, A.F., Chang, T.H., Benchimol, M., Klumpp, D.J., Lehker, M.W., Alderete, J.F. Iron and contact with host cells induce expression of adhesins on surface of *Trichomonas vaginalis*. *Mol. Microbiol.* **47**, 1207–1224, (2003).

[18] Al-Rubaye, F.S.I., Alkhashab, F.M.B. Genotypes diversity and virulence factor screening of *Trichomonas vaginalis* isolated from pregnant women in Mosul (north of Iraq). *Baghdad Sci. J.* **19**, 944–950, doi:10.21123/bsj.2022.6014, (2022).

[19] Nasir, M.A., Al-Idreesi, S.R., Al-Masoudi, W.A. Comparison of different techniques for the diagnosis of *Trichomonas vaginalis* infection in females at reproductive age. *Pak. J. Med. Health Sci.* **16**, 451, (2022).

[20] Al-Tikrity, I.A., Al-Badry, M.S. The trichomonasis spread between married women revisions to the health center in Samarra. *Karbala J. Pharm. Sci.* **5**, 271–276, (2014).

[21] Nasir, M.A., Al-Idreesi, S.R., Al-Masoudi, W.A. Investigation of trichomoniasis among women from Basra Province, Iraq, using the PCR technique. *Int. J. Sci. Eng. Res.* **13**, 1–5, (2022).

[22] Pinto, G.V.S., Bolpet, A.D.N., Martin, L.F., Moço, N.P., Ramos, B.R.A., Silva, M.D.C., et al. Factors associated with *Trichomonas vaginalis* infection in reproductive-aged women attending cervical screening in southeast of Brazil. *Braz. J. Infect. Dis.* **27**, 102794, (2023).

[23] Li, Q., Li, Y., Bai, Y., Zhang, H., Zhao, W. Development and validation of a predictive model for the risk of developing trichomonas vaginitis in women. *Sci. Rep.* **12**, 20182, (2022).

[24] Zhu, X., Liu, L., Yixi, L., Yang, Y., Zhang, Y., Yang, Z., et al. The prevalence and risk factors of *Trichomonas vaginalis* in Wuhan and the Tibetan area, China: A two-center study. *Parasitol. Res.* **122**, 265–273, (2023).

[25] Mahdi, N.K., Gany, Z.H., Sharief, M. Risk factors for vaginal trichomoniasis among women in Basra, Iraq. *East. Mediterr. Health J.* **7**, 918–924, (2001).

[26] Al-Abodi, H.R.J. Suspicion in the form of infection is the basis for selecting the appropriate method for examining the toxoplasmosis disease of bends that have no symptoms from patients. *Int. J. Adv. Res.* **6**, 655–662, (2018).

[27] Alderete, J.F. The *Trichomonas vaginalis* phenotypically varying P270 immunogen is highly conserved except for numbers of repeated elements. *Microb. Pathog.* 27, 93–104, (1999).

[28] Kadir, M.A.-A., Rafik, H.N. Biochemical parameters in patients with *Trichomonas vaginalis* and *Toxoplasma gondii* in Erbil-Iraq. *Tikrit J. Pharm. Sci.* **9**, 327–337, (2013).

[29] Kadir, M., Sulyman, M., Dawood, I., Shams-Eldin, S. *Trichomonas vaginalis* and associated microorganisms in women with vaginal discharge in Kerkuk-Iraq. *Ankara Med. J.* **14**, 91–99, (2014).