Tehran University of Medical Sciences Publication
http://tums.ac.ir

Iran J Parasitol

Open access Journal at
http://ijpa.tums.ac.ir

Iranian Society of Parasitology
http://isp.tums.ac.ir

Original Article

Ferredoxin Gene Mutation and Metronidazole Susceptibility in *Trichomonas vaginalis* Isolates from Hamadan, Iran

Kazem Karami [1], Mohammad Fallah [1], Mousa Motavalli Haghi [1], *Mohammad Matini [2]

1. *Department of Medical Parasitology and Mycology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Infectious Disease Research Center, Avicenna Institute of Clinical Sciences, Hamadan University of Medical Sciences, Hamadan, Iran*

Received 14 May 2026
Accepted 20 Sep 2026

Keywords:
Drug resistance;
Ferredoxins;
Iran;
Metronidazole;
Microbial sensitivity tests;
Trichomonas vaginalis

***Correspondence**
Email:
matini@umsha.ac.ir

Abstract

Background: Trichomoniasis is a sexually transmitted infection primarily treated with nitroimidazoles. Driven by increasing concerns about drug resistance, this study evaluated ferredoxin gene mutation and metronidazole susceptibility in *Trichomonas vaginalis* isolates from Hamedan, Iran.

Methods: This study had two components in Hamadan, 2023: a prevalence assessment of 406 specimens and a laboratory-based analysis of 27 *T. vaginalis* isolates (one fresh isolate from current sampling and 26 archival isolates from earlier studies). Parasites were cultured and subjected to in vitro metronidazole susceptibility testing. Molecular analyses were performed to detect the -239A>T mutation in the ferredoxin gene promoter using genomic DNA extracted from parasite cultures.

Results: *T. vaginalis* was identified in only 0.25% (1/406) of clinical specimens through conventional parasitological methods. All isolates were susceptible to metronidazole. Aerobic MLCs significantly decreased from 27.54 ± 22.13 µg/mL at 24 h to 9.12 ± 6.05 µg/mL at 48 h (P<0.001). Of 27 sequenced amplicons, 23 yielded high-quality sequences. One isolate (4.35%) harbored the -239A>T mutation, exhibiting MLCs of 12.5 µg/mL at 24 h and 1.3 µg/mL at 48 h.

Conclusion: Ferredoxin gene mutations showed a low frequency in this region. All isolates, including the sole isolate harboring the −239A>T mutation, were phenotypically susceptible to metronidazole. While these findings cannot definitively rule out the potential role of this mutation in drug resistance, they support the multifactorial nature of metronidazole resistance in *T. vaginalis*. Consequently, despite the current low prevalence of the infection, ongoing surveillance remains essential to detect emerging resistance and guide clinical management.

Copyright © 2026 Karami et al. Published by Tehran University of Medical Sciences.
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International license.
(https://creativecommons.org/licenses/by-nc/4.0/). Non-commercial uses of the work are permitted, provided the original work is properly cited

Introduction

Human urogenital trichomoniasis is a common sexually transmitted infection worldwide, caused by the flagellated protozoan parasite *T. vaginalis*. According to the WHO, the estimated global prevalence of trichomoniasis in 2020 was 4.9% among women and 0.5% among men aged 15–49 years. An estimated 156.3 million new infections occurred globally in this age group during the same year (1). Trichomoniasis is a curable infection that affects most women and is often asymptomatic. Spontaneous remission of untreated trichomoniasis in men has been reported in 36% to 69% of cases, while in women the infection can persist for years without treatment. Trichomoniasis is thought to increase the risk of adverse outcomes in pregnancy, and the infection in men may be accompanied by epididymitis, prostatitis and infertility. However, a major health concern with trichomoniasis is the increased risk of transmission and acquisition of HIV in both sexes (2,3).

5-nitroimidazole compounds are the drug of choice for the treatment of trichomoniasis. Metronidazole, tinidazole, and most recently secnidazole are approved by the United States Food and Drug Administration for the treatment of trichomoniasis. Since 1962, treatment failure with metronidazole, as the first-line drug, has been documented and the cases have been on the rise. Recent literature reviews found that metronidazole resistance in clinical *T. vaginalis* isolates (2.2%–9.6%) is higher than tinidazole (0%–2%). Moreover, the most cases had low (MLC, 50 to 100 µg/ml) to moderate (MLC, 200 µg/ml) resistance to metronidazole and high level resistance (MLC, ≥400 µg/ml) was rare (4-6). In Iran, in vitro drug susceptibility assay was demonstrated that 2% of clinical *T. vaginalis* isolates had low (MLC, 50 µg/ml) level resistance to metronidazole (7). Metronidazole passively enters the cells by simple diffusion as an inactive prodrug and requires reduction in its nitro group to form nitro radical anions which are toxic to the protozoan cells. Several mechanisms have been proposed for the reduction of the nitro group and the activation of metronidazole. In anaerobic microbial metabolism, the most relevant molecules involved in metronidazole reduction are ferredoxin and pyruvate:ferredoxin oxidoreductase (PFOR). Metronidazole is reduced in *Trichomonas* hydrogenosomes, an organelle related to mitochondria, via the energy production pathway and competing with hydrogenase for ferredoxin electrons (8-11). The ferredoxin gene mutation can reduce the expression of ferredoxin in metronidazole-resistant strains of *Trichomonas*. Two single point mutations have been detected in the upstream promoter region of ferredoxin gene, at nucleotide positions −178 and −239. A 23-kDa DNA-binding protein regulates transcription of the ferredoxin gene by binding to the 28-bp region containing the -239 mutation. This mutation leads to less binding affinity to the DNA-binding protein and reduced transcription (12, 13).

Despite the importance of drug resistance mechanisms, there is a distinct lack of data regarding the ferredoxin gene mutation and its relationship with metronidazole susceptibility in Iranian *T. vaginalis* isolates. Although newer mechanisms such as flavin reductase 1 (FR1) deficiency are increasingly recognized in *Trichomonas*, studying a fundamental molecule essential for parasite metabolism, like ferredoxin, and investigating its mutations is crucial for initial epidemiological screening. Therefore, examining this classic marker is essential to establish a comprehensive regional baseline.

For this purpose, we investigated metronidazole susceptibility and the -239 ferredoxin gene mutation in clinical *T. vaginalis* isolates collected from Hamadan city, west of Iran.

Materials and Methods

Study and sample collection

The current study was confirmed by the research ethics committee of the Hamadan University of Medical Sciences and the ethics code IR.UMSHA.REC.1402.100 was assigned to it.

The study was conducted in two distinct phases: 1) a cross-sectional prevalence assessment of fresh clinical specimens, and 2) a laboratory-based susceptibility and molecular analysis of *T. vaginalis* isolates. For the prevalence component, 406 clinical specimens (203 vaginal samples and 203 first-catch urine samples) were collected from women attending the gynecology clinic and clinical laboratory of Atieh Hospital in Hamadan from Apr to Dec 2023. First, informed consent was obtained from all participants. Subsequently, demographic and clinical data were collected using a standardized questionnaire, followed by collection of the clinical samples. The samples were subjected to parasitological method, wet mount microscopy and Dorset culture medium. Then, the isolated parasites subjected to axenic culture in TYI-S-33 medium. For the laboratory-based component, in addition to the single fresh isolate obtained from the current 2023 sampling, we utilized a subset of 26 *T. vaginalis* isolates previously collected and cryopreserved by the Parasitology Research Laboratory of Hamadan University of Medical Sciences. These archival isolates were originally obtained from women attending health and gynecology centers in Hamadan province during four regional epidemiological studies conducted between 2015 and 2022 (14-17). The cryopreserved isolates were revived and axenically grown before drug susceptibility testing and molecular analyses (14).

In vitro drug sensitivity assay

The minimum lethal concentration (MLC) was defined as the lowest concentration of metronidazole which immobilized and killed all trichomonads. Based on published CDC-associated susceptibility data, an aerobic MLC of ≥50 µg/mL has been associated with metronidazole treatment failure; therefore, MLC values <50 µg/mL after 48 h of drug exposure were interpreted as being below the treatment-failure-associated level, serving as a conventional threshold to differentiate susceptible isolates from those with reduced susceptibility (18). Susceptibility testing was performed on 96-well microtiter plates, based on the protocol used by the Centers for Disease Control and Prevention (CDC) (19). Briefly, *Trichomonas* cells were cultured at 10^4 trophozoites per well in sterile microtiter plates in serial 2-fold concentration dilutions of metronidazole (400 to 0.1 µg/ mL). Then, the plates were aerobically incubated at 35.5°C for 24 and 48 h and examined under an inverted microscope. The experiments were performed in duplicate and repeated twice separately (20).

DNA extraction, amplification and sequencing

Trichomonas cells were subjected to DNA extraction using a commercial extraction kit (DNG plus DNA Extraction Kit, Cinacloon, Tehran, Iran) following the manufacturer's protocol. Concentration and purity of extracted DNA were evaluated by NanoDrop 1000 spectrophotometer. The *Trichomonas* ferredoxin gene was amplified with specific primers, TVf Forward: 5´ CGACAGATAATAATTTTTTTTGAAAA 3´TVf Reverse: 5´-TGCAGATGCACTTGCCGC - 3´ (13). Amplification of the target gene was performed in a total volume of 25 ml containing 1 ng of DNA, 20 picomol of reverse and forward primers, 1.5 unit of Taq DNA polymerase, 0.2 mM dNTP, 1.5 mM MgCl2 and 1X specific PCR buffer. PCR was carried out in 40 cycles with the following condition: initial denaturation at 94 °C for 5 min, denaturation step at 94 °C for 30 sec, primer annealing step at 46 °C for 1 min, extension step at 72 °C for 30 sec, and final extension 72 °C for 7 min. After amplification, PCR products were visualized by SYBR Safe stain on a 1% agarose gel electrophoresis. The PCR amplicons of ferredoxin gene were purified and were subjected to sequencing by automated DNA sequencer ABI 3500 (Codon Genetic Group, Tehran, Iran). The obtained sequences were edited using Chromas (ver. 2.6) and analyzed using CLC Genomics Workbench software (ver. 3.6.5). Only sequences exhibiting clear, unam-

biguous chromatogram peaks were retained for analysis. Four isolates (Tv6, Tv7, Tv10, and Tv12) were excluded from analysis because their amplicons produced poor-quality, noisy chromatograms. Multiple sequence alignment was performed using the online Multalin tool (http://multalin.toulouse.inra.fr/multalin/). The retained high-quality consensus sequences were then compared by aligning the query sequences against *Trichomonas* ferredoxin gene sequences using the BLASTn program (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) in the GenBank database. The nucleotide sequences generated in the present study were not deposited in GenBank, and therefore no accession numbers are available for these newly generated sequences.

Data analysis

Statistical analysis of data was performed using SPSS ver. 23. (IBM Corp., Armonk, NY, USA). The *P*-value<0.05 was applied to be detected as significant.

Results

Participants

Among 406 clinical specimens examined, only one urine sample (0.25%) tested positive for *T. vaginalis* using standard parasitological methods. The participants ranged in age from 20 to 56 yr, with a mean (±SD) age of 32.46 ± 6.28 yr. All the women were married and presented with vaginal discharge. Other reported symptoms included genital irritation/itching (97.8%), dyspareunia (14.8%), dysuria (14.6%), frequent urination (8.9%), and lower abdominal pain (8.4%). Notably, the *T. vaginalis*-positive case did not report painful urination, urinary frequency, or lower abdominal pain. Complete demographic characteristics of the participants are presented in Table 1.

Table 1: Frequency of trichomoniasis and demographic information of women attending the Atieh Hospital in Hamadan, 2023

Variables	*Trichomonal vaginitis* Infection			*P* value
	Positive n (%)	Negative n (%)	Total n (%)	
Age (yr) 21-29 30-39 40≤	0 (0) 1 (0.5) 0 (0)	178 (100) 196 (99.5) 31 (100)	178 (43.8) 197 (48.5) 31 (7.7)	1
Education Illiterate Primary school Junior high school College education	0 (0) 0 (0) 1 (0.5) 0 (0)	10 (100) 47 (100) 202 (99.5) 146 (100)	10 (2.5) 47 (11.6) 203 (50) 146 (35.9)	0.836
Occupation Homemaker Employee Student Other	1 (0.3) 0 (0) 0 (0) 0 (0)	360 (99.7) 13 (100) 24 (100) 8 (100)	361 (88.9) 13 (3.2) 24(5.9) 8 (2)	1
Residency Urban Rural	0 (0) 1 (6.3)	390 (100) 15 (93.7)	390 (96.1) 16 (3.9)	0.039
Contraceptive methods Oral contraceptive pills Condoms Other None	0 (0) 1 (0.4) 0 (0)) 0 (0)	72 (100) 276 (99.6) 16 (100) 41 (100)	72 (17.7) 277 (68.3) 16 (3.9) 41 (10.1)	0.521

Drug susceptibility

For the laboratory-based component, 27 *T. vaginalis* isolates (comprising the single fresh isolate from the current 2023 sampling and 26 archival isolates from previous regional studies) were successfully cultured and subjected to drug susceptibility testing. Under aerobic conditions, the 24-hour MLC values of iso-

lates ranged from 3.1 to 100 µg/mL with an average (±SD) of 27.54 ± 22.13 µg/mL. After 48 h of exposure, the response to metronidazole increased (P<0.001). The average (±SD) 48-hour MLC for metronidazole was 9.12 ± 6.05 µg/mL with a range of 1.6 to 25 µg/mL (Fig. 1). The results of 48-hour susceptibility assay showed that all *Trichomonas* isolates were susceptible to metronidazole.

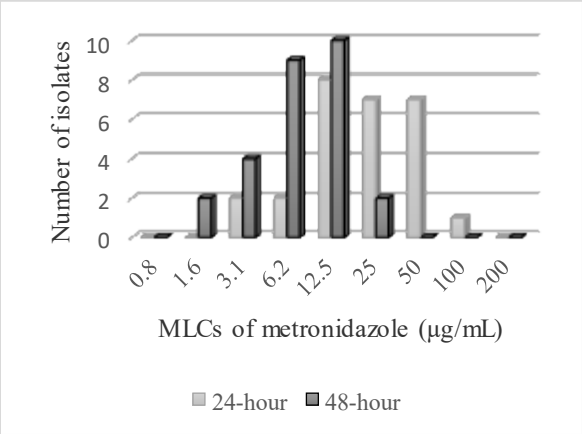


Fig. 1: Aerobic Drug susceptibility testing of *Trichomonas vaginalis* isolates. MLC (Minimum Lethal Concentration) is defined as the lowest drug concentration that immobilizes and kills all trophozoites

Molecular analysis

PCR reaction was performed on 27 DNA *Trichomonas* samples, successfully amplifying a 456-bp fragment of the ferredoxin gene in all specimens (Fig. 2). The PCR amplicons were sequenced, with 23 high-quality sequences retained for downstream analysis. Two sequence types (haplotypes), designated TvH1 (n=22) and TvH2 (n=1), were identified through sequence alignment. The TvH1 and TvH2 haplotype sequences exhibited 100% identity when aligned with their respective reference sequences: the wild-type (GenBank accession: JQ969043) and the mutant (GenBank accession: JQ969042) (Fig. 3). BLAST analysis of the sequences in the GenBank database demonstrated that these sequences were >99%–100% homologous to the *T. vaginalis* ferredoxin gene. In only one isolate (4.35%), the Tv5 sequence (haplotype TvH2) was found to carry a point mutation (A→T) at nucleotide position −239 (Fig. 3). A detailed schematic representation of this specific substitution within the ferredoxin promoter region is illustrated in Fig. 4.

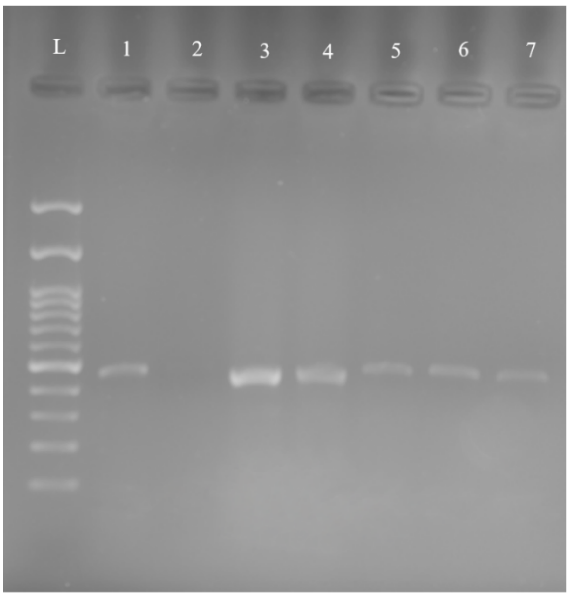


Fig. 2: Agarose gel electrophoresis of PCR-amplified 456-bp ferredoxin gene fragment from *T. vaginalis* isolates. Lane L: 100-bp DNA ladder; Lane 1: Positive control; Lane 2: Negative control; Lanes 3-7: Isolates. The 456-bp fragment corresponds to the promoter region of the ferredoxin gene

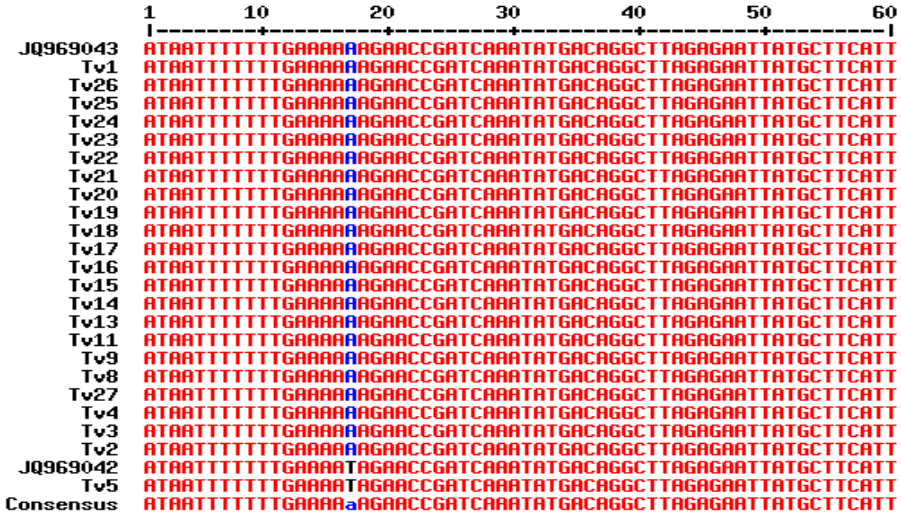


Fig. 3: Multiple sequence alignment of the partial ferredoxin gene promoter region in *Trichomonas vaginalis* isolates. Nucleotide sequences of 24 Iranian *T. vaginalis* isolates were aligned with wild-type (JQ969043) and mutant (JQ969042) reference sequences from GenBank. The alignment spans 60 nucleotides of the promoter region upstream of the translation start codon. The consensus sequence and conservation profile are shown at the bottom. The Tv5 isolate exhibits a nucleotide substitution at position corresponding to −239, matching the mutant reference sequence JQ969042, while all other isolates are identical to the wild-type reference JQ969043

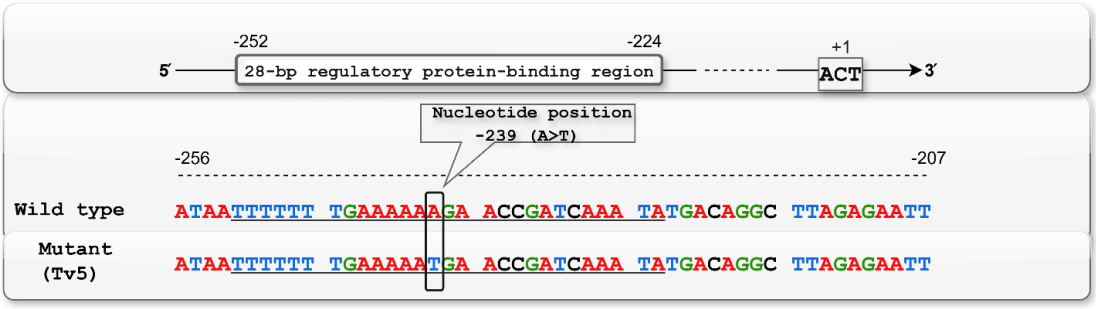


Fig. 4: Detailed representation of the ferredoxin promoter region showing the −239A>T substitution in *Trichomonas vaginalis*. The nucleotide sequences of the wild-type and mutant (Tv5) isolates are aligned, and nucleotide position −239 is indicated relative to the transcription start site (+1). The A nucleotide present at position −239 in the wild-type sequence is replaced by T in the mutant Tv5 sequence. The location of the 28-bp regulatory protein-binding region is indicated above the alignment

Discussion

Although all women experienced vaginal discharge and many showed additional symptoms of vaginitis, just one case was linked to trichomoniasis, indicating that other microbial agents were more prevalent causes of vaginitis in this population. Globally, the most common causes of vaginitis are trichomoniasis, bacterial vaginosis, and vulvovaginal candidiasis. Bacterial vaginosis has a particularly high prevalence worldwide, with studies estimating rates of 23% to 29% across various regions (21). A 2021 study in northern Iran found a notably higher prevalence of vulvovaginal candidiasis (38.5%) than bacterial vaginosis (5.8%) and trichomoniasis (5.0%) (22). Diagnosing the causes of vaginitis based only on clinical symptoms is difficult because symp-

toms often overlap between different types. Therefore, laboratory testing is essential for accurately identifying the pathogen and ensuring effective treatment.

Meta-analyses and systematic reviews conducted in Iran across two different time frames (1999–2012 and 2000–2023) reported trichomoniasis prevalence rates of 8.0% and 4.3%, respectively. The observed reduction in prevalence likely reflects improvements in public health infrastructure, increased availability of healthcare services, and the implementation of broader health education initiatives within community (23, 24). Supporting these findings, recent epidemiological investigations in Hamadan province (2011–2022) reported *T. vaginalis* prevalence ranging from 0.5% to 2.4% (16, 17, 25). In the current study, the frequency is even lower than earlier regional estimates, further reinforcing a sustained downward trend in infection rates within the population. Consequently, due to this extremely low overall frequency, the statistical power of our dataset is limited. For instance, while a statistically significant *P*-value (*P*=0.039) was observed for residency in Table 1, this finding is based on a single positive case (1/16 rural vs. 0/390 urban). Therefore, no reliable epidemiological conclusions regarding residency or other demographic risk factors can be drawn from this specific dataset.

The increasing prevalence of metronidazole-resistant trichomoniasis has become a significant public health challenge, largely due to the absence of clinically effective treatment options outside the nitroimidazole class. In vitro drug susceptibility studies have documented geographical differences in metronidazole resistance among *T. vaginalis* clinical isolates, with reported resistance rates of 4.3%–10% in the United States and 17.4% in New Guinea (26, 27).

Metronidazole resistance in *Trichomonas* is a multifactorial phenomenon involving diverse mechanisms, such as impaired drug uptake, altered drug activation pathways, and metabolic adaptations. Although certain mechanisms—particularly anaerobic resistance in laboratory-induced strains—are well characterized, the complete spectrum of clinical resistance remains incompletely understood (5, 10, 28).

Quon et al. established a significant association between diminished ferredoxin levels and metronidazole resistance in *T. vaginalis* (12). Their findings revealed that resistant strains exhibit downregulated ferredoxin gene expression and reduced mRNA translation compared to susceptible strains. This impaired ferredoxin synthesis likely contributes to resistance by diminishing the parasite's capacity to activate metronidazole into its cytotoxic form. The observed transcriptional downregulation may stem from a specific -239 nucleotide mutation that attenuates binding affinity of the 23-kDa transcriptional regulator, ultimately suppressing ferredoxin expression (12). The role of ferredoxin in metronidazole resistance in *Trichomonas* remains unclear. While some studies suggest ferredoxin may contribute to nitroimidazole sensitivity (29), other experimental evidence demonstrates that targeted inhibition of the ferredoxin gene in vitro fails to induce metronidazole resistance (30). While ferredoxin downregulation (particularly ferredoxin 1 and 2) is observed in laboratory-induced resistant strains, this pattern is absent in clinically resistant isolates. These observations fundamentally contradict the established model of metronidazole activation through ferredoxin-dependent electron transfer. Instead, the studies highlights the pivotal role of FR1 as a ferric iron reductase ($Fe^{3+} \rightarrow Fe^{2+}$), facilitating non-enzymatic metronidazole activation through ferrous iron (Fe^{2+}) production. Notably, FR1 deficiency is consistently associated with resistance in both laboratory and clinical strains, disrupting the formation of cytotoxic iron-cysteine-metronidazole complexes required for drug activation. Particularly in clinical settings, metronidazole resistance may arise primarily from disruptions in iron metabolism rather than canonical ferredoxin-

mediated electron transfer. This study redefines the previous framework of metronidazole resistance, shifting focus from ferredoxin-dependent pathways to FR1-regulated iron redox homeostasis. This paradigm underscores the need to explore iron-targeting strategies for overcoming resistance in Trichomonas vaginalis (31).

In our study, all *Trichomonas* isolates were drug-susceptible. A -239A>T point mutation was identified in one isolate (Tv5), which exhibited MLCs of 12.5 µg/mL at 24 h and 1.3 µg/mL at 48 h. In the study by Heidari et al., 8.69% (4/46) of *T. vaginalis* isolates from Tehran Province carried this mutation. However, this study did not include in vitro drug susceptibility testing data for the analyzed clinical isolates (13). To date, only a single *T. vaginalis* isolate exhibiting reduced metronidazole susceptibility (MLC = 50 µg/mL) has been documented in Iran through standardized in vitro drug susceptibility testing (7). Comprehensive susceptibility testing of Iranian clinical isolates consistently demonstrates maintained metronidazole efficacy, with MLC values spanning 0.1-25 µg/mL across studies (14, 32). In the present study, all tested isolates, including the sole isolate harboring the −239A>T mutation, were phenotypically susceptible to metronidazole. Although our findings cannot definitively rule out the potential role of this mutation in drug resistance, they provide further evidence supporting the multifactorial nature of metronidazole resistance in *T. vaginalis*, which likely involves complex interactions between multiple genetic and metabolic pathways rather than a single point mutation. Consequently, further molecular epidemiological studies are required to determine the true prevalence and clinical significance of this mutation across different geographical regions.

A limitation of this study is the extremely low clinical prevalence observed (only one positive case out of 406), which restricted our ability to draw robust epidemiological associations. Additionally, our reliance on in vitro susceptibility testing without direct clinical outcome correlation, combined with the small sample size of successfully sequenced isolates (n=23), limits the generalizability of our molecular findings. While this study prioritized the ferredoxin -239 mutation as a foundational marker to establish a regional epidemiological baseline, we acknowledge that emerging mechanisms like FR1 deficiency also play a critical role in clinical resistance. Finally, due to the extremely low yield of *T. vaginalis* in the current clinical sampling (yielding only a single fresh isolate in 2023), the inclusion of 26 archival isolates collected between 2015 and 2022 was necessary to achieve a sufficient sample size for phenotypic and molecular analyses. Consequently, our dataset represents a broader temporal and regional baseline rather than exclusively the 2023 population of Hamadan city.

Conclusion

The present study revealed a low frequency of trichomoniasis in the study population, despite the persistent clinical significance of vaginitis symptoms among affected individuals. Additionally, ferredoxin gene mutations were found to be rare among the local *T. vaginalis* isolates. Importantly, all tested isolates, including the sole isolate harboring the −239A>T mutation, were phenotypically susceptible to metronidazole. Although metronidazole-resistant strains were not identified and the exact role of this mutation cannot be definitively ruled out, our findings provide evidence supporting the multifactorial nature of metronidazole resistance in *T. vaginalis*, highlighting the need for further large-scale studies. These results advocate for sustained antimicrobial resistance surveillance programs employing dual methodological approaches: phenotypic characterization through standardized in vitro drug susceptibility assays and genotypic analysis using established molecular markers. Such integrated monitoring is crucial for detecting

resistance evolution and informing therapeutic strategies.

Acknowledgements

The authors extend their sincere gratitude to all participants involved in this study, as well as to the clinicians at Atieh Hospital for their invaluable cooperation. Additionally, the authors acknowledge the financial support provided by the Vice Chancellor for Research and Technology at Hamadan University of Medical Sciences (Grant No.: 140203232126).

Conflict of interest

The authors declare no conflict of interests.

References

1. World Health Organization. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021: accountability for the global health sector strategies 2016–2021. Geneva: World Health Organization; 2021. Web Annex 1: Key data at a glance. Global and regional estimates of four sexually transmitted infections, 2020. Available from: https://iris.who.int/bitstream/handle/10665/342808/9789240030985-eng.pdf
2. Kissinger PJ, Gaydos CA, Seña AC, et al. Diagnosis and Management of *Trichomonas vaginalis*: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections Treatment Guidelines. Clin Infect Dis. 2022; 13;74(Suppl_2):S152-S161.
3. Van Gerwen OT, Opsteen SA, Graves KJ, et al. Trichomoniasis. Infect Dis Clin North Am. 2023; 37(2):245-265.
4. Muzny CA, Van Gerwen OT, Legendre D. Secnidazole: a treatment for trichomoniasis in adolescents and adults. Expert Rev Anti Infect Ther. 2022; 20(8):1067-1076.
5. Graves KJ, Novak J, Secor WE, et al. A systematic review of the literature on mechanisms of 5-nitroimidazole resistance in *Trichomonas vaginalis*. Parasitology. 2020; 147(13):1383-1391.
6. Marques-Silva M, Lisboa C, Gomes N, et al.. *Trichomonas vaginalis* and growing concern over drug resistance: a systematic review. J Eur Acad Dermatol Venereol. 2021; 35(10):2007-2021.
7. Matini M, Maghsood AH, Mohebali M, et al. In Vitro Susceptibility of Iranian Isolates of *Trichomonas vaginalis* to Metronidazole. Iran J Parasitol. 2016; 11(1):46-51.
8. Leitsch D. A review on metronidazole: an old warhorse in antimicrobial chemotherapy. Parasitology. 2019; 146(9):1167-1178.
9. Huang PJ, Huang CY, Li YX, et al. Dissecting the Transcriptomes of Multiple Metronidazole-Resistant and Sensitive *Trichomonas vaginalis* Strains Identified Distinct Genes and Pathways Associated with Drug Resistance and Cell Death. Biomedicines. 2021; 9(12):1817.
10. Bradic M, Warring SD, Tooley GE, et al. Genetic Indicators of Drug Resistance in the Highly Repetitive Genome of *Trichomonas vaginalis*. Genome Biol Evol. 2017; 9(6):1658-1672.
11. Xie Y, Zhong P, Guan W, et al. Transcriptional profile of *Trichomonas vaginalis* in response to metronidazole. BMC Genomics. 2023; 24(1):318.
12. Quon DV, d'Oliveira CE, Johnson PJ. Reduced transcription of the ferredoxin gene in metronidazole-resistant *Trichomonas vaginalis*. Proc Natl Acad Sci U S A. 1992; 89(10):4402-6.
13. Heidari S, Bandehpour M, Seyyed-Tabaei SJ, et al. Ferredoxin Gene Mutation in Iranian *Trichomonas vaginalis* Isolates. Iran J Parasitol. 2013; 8(3):402-7.
14. Matini M, Rezaei H, Fallah M, et al. Genotyping, Drug Susceptibility and Prevalence Survey of *Trichomonas vaginalis* among Women Attending Gynecology Clinics in Hamadan, Western Iran, in 2014-2015. Iran J Parasitol. 2017; 12(1):29-37.
15. Akbari Z, Matini M. The Study of Trichomoniasis in Pregnant Women

Attending Hamadan City Health Centers in 2015. Avicenna J Clin Microbiol Infect. 2017; 4(2):e41533.
16. Morshedloo L, Fallah M, Maghsood AH, et al. Study of *Trichomonas vaginalis* Infe-ction in Women Visiting Health Centers in Bahar City and Determination of Metronidazole Susceptibility of the Isolated Parasites. Avicenna J Clin Med. 2018; 24(4): 315-321.
17. Azimi Kohn A, Foroughi-Parvar F, Matini S, et al. Prevalence of trichomoniasis in women attending health centers in Asadabad city, West of Iran, during 2021-2022. Avicenna J Clin Microbiol Infect. 2024; 11(1):1-4.
18. Centers for Disease Control and Prevention. *Trichomonas vaginalis* Susceptibility Testing. Atlanta: CDC; (cited 2026 Sep 4). Available from: https://www.cdc.gov/parasites/hcp/laboratories/trichomonas-vaginalis-susceptibility-testing.html
19. Schwebke JR, Barrientes FJ. Prevalence of *Trichomonas vaginalis* isolates with resistance to metronidazole and tinidazole. Antimicrob Agents Chemother. 2006; 50(12):4209-10.
20. Karami F, Dastan D, Fallah M, et al. In vitro antitrichomonal activity of Satureja khuzestanica and main essential oil components carvacrol, thymol, and eugenol. J Infect Dev Ctries. 2023; 17(1):80-85.
21. Peebles K, Velloza J, Balkus JE, et al. High Global Burden and Costs of Bacterial Vaginosis: A Systematic Review and Meta-Analysis. Sex Transm Dis. 2019; 46(5):304-311.
22. Salehi M, Minooeianhaghighi M, Mohammadzadeh A, et al. Prevalence and Factors Related to Trichomoniasis, Candidal Vaginitis, and Bacterial Vaginosis in Northeast Iran. Int J Microbiol. 2025; 2025:9573665.
23. Hezarjaribi HZ, Fakhar M, Shokri A, et al. *Trichomonas vaginalis* infection among Iranian general population of women: a systematic review and meta-analysis. Parasitol Res. 2015; 114(4):1291-300.
24. Moghadamizad Z, Khalili JY, Olfatifar M, et al. The prevalence of *Trichomonas vaginalis* infection among the female population of Iran: a systematic review and meta-analysis. Int Health. 2024; 16(3):240-251.
25. Matini M, Rezaie S, Mohebali M, et al. Prevalence of *Trichomonas vaginalis* Infection in Hamadan City, Western Iran. Iran J Parasitol. 2012;7(2):67-72.
26. Kissinger PJ, Gaydos CA, Seña AC, et al. Diagnosis and Management of *Trichomonas vaginalis*: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections Treatment Guidelines. Clin Infect Dis. 2022; 74(Suppl_2):S152-S161.
27. Upcroft JA, Dunn LA, Wal T, et al. Metronidazole resistance in *Trichomonas vaginalis* from highland women in Papua New Guinea. Sex Health. 2009; 6(4):334-338.
28. Leitsch D. Recent advances in the molecular biology of the protist parasite *Trichomonas vaginalis*. Fac Rev. 2021; 10:26.
29. Vidakovic M, Crossnoe CR, Neidre C, et al. Reactivity of reduced [2Fe-2S] ferredoxins parallels host susceptibility to nitroimidazoles. Antimicrob Agents Chemother. 2003; 47(1):302-8.
30. Land KM, Delgadillo-Correa MG, Tachezy J, et al. Targeted gene replacement of a ferredoxin gene in *Trichomonas vaginalis* does not lead to metronidazole resistance. Mol Microbiol. 2004; 51(1):115-22.
31. Mayr AL, Paunkov A, Hummel K, et al. Comparative proteomic analysis of metronidazole-sensitive and resistant *Trichomonas vaginalis* suggests a novel mode of metronidazole action and resistance. Int J Parasitol Drugs Drug Resist. 2024; 26:100566.
32. Fallah M, Mosayebi M, Matini M, et al. Prevalence of Trichomoniasis and Determination of in Vitro Susceptibility of Isolated Parasites to Metronidazole in Women Referred to Health Centers in Arak, Iran, in 2020. Avicenna J Clin Med. 2021; 28(3):186-193.