

A Comparison Between Bacterial and Trichomonas Infection Isolated from Women of Childbearing and Menopausal Age

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Abstract

Trichomonas vaginalis is a protozoan parasitic pathogen responsible for trichomoniasis, a common STD causing vaginal microbiota changes. Overgrowth of *Gardnerella vaginalis*, anaerobic bacteria, and mycoplasmas causes bacterial vaginosis, leading to vulvovaginal symptoms globally. This study aimed to investigate the possibility of vaginal microbiota changes and polymicrobial vaginosis associated with *Trichomonas vaginalis* and to estimate the correlation of some epidemiological factors with trichomoniasis in women of childbearing age in Kirkuk Governorate. This study was conducted in Kirkuk Governorate between November 10, 2023, and March 15, 2024. The total number of married, non-pregnant women was 100, ranging in age from 18 to 45 years. From each participant, two vaginal discharge specimens were collected: identify *Trichomonas vaginalis* via wet-mount microscopy and the other specimens were inoculated into various culture media to isolate aerobic and anaerobic bacteria, or *Candida* spp. A total of 100 examined women (10%) had a *Trichomonas vaginalis* infection. The highest frequency was observed in the 25-29 age group. The study revealed a higher prevalence of *T. vaginalis* infection in rural areas than in urban areas. It was revealed that there was a high rate of infection with *T. vaginalis* among women with low education levels, whereas infection rates decreased as the education level of women increased. This study found that *Gardnerella vaginalis* and *Staphylococcus epidermidis* had the highest association with *Trichomonas vaginalis* infection. The highest prevalence of *T. vaginalis* infection was associated with younger married women, women residing in rural areas, and women with low educational attainment. Trichomoniasis affects the vaginal microbiota by reducing Lactobacilli and has a strong relationship with *Gardnerella vaginalis* and *Staphylococcus epidermidis*.

Keywords: *Trichomonas vaginalis*, Vaginal microbiota, *Gardnerella vaginalis*, Bacterial vaginosis, Vaginal discharge

Introduction

Trichomoniasis is the most common nonviral sexually transmitted disease (STD) in the world. It is caused by the flagellated, anaerobic, parasitic protozoan *Trichomonas vaginalis*. This infection is not a potentially harmful condition that can last for years without a diagnosis and it is frequently transmitted by an asymptomatic carrier (1). The only known host of trophozoites is humans, and they transmit primarily through vaginal sexual contact and sporadically through bites. Around the world, the prevalence of *T. vaginalis* varies between 2% and >50%, depending on the region, nation, sex, and demography of the population under investigation (2). It is estimated that 276.4 million new infections are acquired worldwide each year, leading to dangerous infections of the reproductive tract that primarily affect people of color and those who reside in underprivileged or impoverished areas. One of the five parasitic infections that go untreated in the US is *T. vaginalis* (3).

In contrast to vaginal infections caused by a single pathogen, bacterial infections (BV) are understood as a collection of common clinical signs and symptoms that can be triggered by a wide variety of bacterial species and communities. Various bacterial species exhibit varying associations with presenting signs and symptoms, accounting for a significant degree of variability in clinical presentation (4, 5). The most frequent reason for abnormal vaginal discharge in women of reproductive age is a vaginal dysbiosis called BV (6, 7). Conversely, the presence of characteristics that characterize bacterial vaginosis, such as higher pH (8), loss of facultative lactobacilli, and elevated amine synthesis, increases the colonization of *T. vaginalis*. Certain types of bacteria linked to bacterial vaginosis can form symbiotic relationships with *T. vaginalis* (9). Such bacteria exist in trichomonad isolates from various geographical locations. While *T. vaginalis* and some other types of bacteria can cause disease in the vagina on their own, their combination has been demonstrated to have significant effects on the pathogenicity of each of the other bacteria (10).

Materials & Methods

This cross-sectional study evaluated the effect of trichomoniasis on polymicrobial vaginosis and microbiota changes in childbearing age women; it was carried out between November 10, 2023 and March 15, 2024. The total number of non-pregnant married women who were under study was 100; their ages ranged from 18 to 45 years, and they were selected from those who attended the Azadi Teaching Hospital, Kirkuk General Hospital, and Gynecology and Pediatric Hospital.

The inclusion criteria were child bearing age, non-pregnant married women, who have not had a hysterectomy and who have not been diagnosed with HIV and they were not treated with antibiotics or used vaginal cream for at least 4 weeks of the study.

Vaginal discharge was obtained after placing the patient in a lithotomy position and using a sterile speculum to open the vagina; two specimens of vaginal discharge were collected from the posterior vaginal fornix using sterile vaginal swabs. The goal of this study was to identify motile *Trichomonas vaginalis* through a wet-mount smear within 1 h. Approximately 1mL of normal saline was added to the swab tube to maintain the parasite's vitality and increase its moisture for microscopic examination.

To identify polymicrobial vaginosis, the second swab was inoculated into diverse bacteriological media, like MacConkey agar, chocolate agar, and blood agar to isolate aerobic and anaerobic bacteria or *Candida* spp. The media for aerobic bacteria were incubated at 37°C for 24-72 hours, while the media for anaerobic bacteria were incubated for 48 h at 37°C in an anaerobic environment containing 3-5% carbon dioxide and supplied by candles (Candle Jar). Biochemical tests including catalase, coagulase, urease, and oxidase were used to identify and differentiate the cultivated isolates.

Results & Discussion

The study included 100 women, of whom 10 (10%) were diagnosed with *T. vaginalis* infection. The mean age of participants was 29.5±11.74 years. The highest percentage of individuals who tested positive for *T. vaginalis* in this study fell within the age group of 25–29 years, with 4 patients, or 15.4% of the total group, followed by the age group of 18–24 years, with 3 patients, or 13% of the total group. The lowest percentage of positive *T. vaginalis* tests was in the 40–45 age group (Table 1). Our findings are consistent with those previous studies demonstrating that *T. vaginalis* infection is more common in younger women (11). This age-related pattern may be attributed to several factors, including: Behavioral factors: Younger women may engage in riskier sexual behaviors, such as having multiple partners and inconsistent condom use, increasing their likelihood of exposure to *T. vaginalis* (12). Biological factors: Changes in vaginal pH and vaginal microbiota composition is associated with age can influence susceptibility to *T. vaginalis* infection (13). However, our finding of no infections in the 40-45 age group contrasts with some studies reporting persistent *T. vaginalis* prevalence in older women (14) (Table 1).

Table 1. Relationship of *Trichomonas vaginalis* infection with age.

<i>Age group</i>	<i>No. of women tested for T. vaginalis</i>	<i>No. of positive cases</i>	<i>Percentage</i>
18-24	23	3	13%
25-29	26	4	15.4%
30-34	20	2	10%
35-39	18	1	5.5%
40-45	13	0	0%
All participants	100	10	10%

In this study, rural areas showed a higher prevalence rate than urban areas, as the prevalence of *T. vaginalis* infection in urban areas was 4%, whereas that in rural areas was 6%. Statistical analysis showed no significant association ($P = 0.50286$) between patient residence and the prevalence of *T. vaginalis* infection (Table 2). This finding contradicts several studies reporting a higher prevalence of STIs, including *T. vaginalis*, in rural populations. For instance, a study (15) found a significantly higher prevalence of *T. vaginalis* in rural women than urban women, attributing it to factors like limited healthcare access, as highlighted by (16), who demonstrated the impact of limited healthcare access on STI rates in rural communities. In addition, cultural factors such as sexual health stigma in rural settings, as discussed by (17), may discourage people from undergoing testing and receiving treatment, further contributing to infection rates. As in Table 2.

Table 2. The distribution of infection by *Trichomonas vaginalis* parasite according to area of residence.

<i>Residence</i>	<i>No. of women tested for T. vaginalis</i>	<i>No. of positive cases</i>	<i>Percentage</i>	<i>P Value</i>
Rural	50	6	12%	0.50286
urban	50	4	8%	
Total	100	10	10%	

The results of the study showed a high rate of infection with *T. vaginalis* among women with an illiterate educational level of 4%, followed by participants in primary and secondary schools (2%) each, and 1% among women with institute education and university or higher education levels (Table 3). These results align with previous research suggesting a link between lower educational attainment and increased risk of STIs, including *T. vaginalis*. For example, a study by (18) found that women with less than a high school education were significantly more likely to test positive for *T. vaginalis* than those with higher education levels. This association can be attributed to several factors. Lower educational attainment often correlates with limited access to healthcare, including sexual health services, leading to delayed diagnosis and treatment (19). Additionally, socioeconomic disadvantages, frequently associated with lower educational levels, can increase vulnerability to STIs due to factors like limited access to quality healthcare, reduced health literacy, and increased engagement in risky sexual behaviors (20) (Table 3).

Table 3. Relationship of infection with the *Trichomonas vaginalis* parasite to educational level.

<i>Education level</i>	<i>No. of women tested for T. vaginalis</i>	<i>No. of positive cases</i>	<i>Percentage</i>
Illiterate	28	4	4%
Primary school	13	2	2%
Secondary school	8	2	2%
Institute	19	1	1%

Collage and higher	38	1	1%
Total	100	10	10%

100 women across different age groups, with a focus on those testing positive for *Trichomonas vaginalis* (*T. vaginalis*). The overall prevalence of *T. vaginalis* was 10%, consistent with previous research on similar populations (21). Across all age groups, *Lactobacillus* and *Staphylococcus* species were the predominant microorganisms identified. The presence of *Gardnerella vaginalis* was observed in the 18-24 and 35-39 age groups, both of which tested positive for *T. vaginalis*. This observation aligns with previous studies highlighting the association between *G. vaginalis* and *T. vaginalis* infection, which are often linked to bacterial vaginosis (BV) (22) as Table (4).

Table 4. Age-Related Variations in Vaginal Microbiota Composition in Women with *Trichomonas vaginalis* Infection

Age group	No. of women tested for <i>T. vaginalis</i>	TV Positive group No.	No. of microorganisms isolated	Percentage
18-24	23	3	<i>Lactobacilli</i> (60%), <i>Staph aureus</i> (33%), <i>E. coli</i> (13%), <i>Gardnerella vaginalis</i> (3%)	13%
25-29	15	3	<i>Lactobacilli</i> (60%), <i>Staph aureus</i> (33%), <i>Strep. agalactiae</i> (2%), <i>E. coli</i> (12%), <i>Staph. Epidermidis</i> (13%),	20%
30-34	11	1	<i>Lactobacilli</i> (51%), <i>Staph aureus</i> (43%), <i>E. coli</i> (13%), <i>Proteus spp</i> (2%)	9.10%
35-39	13	1	<i>Lactobacilli</i> (49%), <i>Staph aureus</i> (19%) <i>E. coli</i> (10%), <i>Gardnerella vaginalis</i> (4%) <i>Staph. Epidermidis</i> (11%)	7.70%
40-44	10	1	<i>Lactobacilli</i> (43%), <i>Staph aureus</i> (12%) <i>E. coli</i> (15%), <i>Staph. Epidermidis</i> (11%)	10%
45-49	13	1	<i>Lactobacilli</i> (43%), <i>Staph aureus</i> (13%) <i>E. coli</i> (19%), <i>Staph. Epidermidis</i> (11%)	7.70%
50-54	9	0	<i>Lactobacilli</i> (39%), <i>Staph aureus</i> (17%) <i>E. coli</i> (17%), <i>Staph. Epidermidis</i> (10%)	0%
55-59	6	0	<i>Lactobacilli</i> (33%), <i>Staph aureus</i> (20%) <i>E. coli</i> (21%), <i>Proteus spp</i> (3%) <i>Staph. Epidermidis</i> (10%)	0%
All participants	100	10		10%

The clinical characteristics of the participants in the study are displayed in Table 5. Out of 100 participants, 57 (57%) women reported abnormal vaginal discharge; among the TV- positive group, there were 9 (9%) women revealed abnormal vaginal discharge, whereas 48 (48%) of the TV- negative group who demonstrated abnormal vaginal discharge. These findings revealed significant association between abnormal vaginal discharge and the presence of *T. vaginalis* infection ($P = 0.02642$). This finding is consistent with that of previous research showing that abnormal vaginal discharge is a common symptom of trichomoniasis (23). The higher prevalence of *Lactobacillus* species (55%) in women with abnormal discharge and TV infection may be related to the organism's ability to produce lactic acid, which contributes to the acidic environment often associated TV infection (24). Although other clinical characteristics, such as genital itching, sores, redness, swelling, and dyspareunia, were reported in both TV-positive and TV-negative women, no statistically significant associations were observed. This may reflect the multifactorial nature of these symptoms, which can be caused by a various condition besides trichomoniasis (25).

Table 5. Association of Abnormal Vaginal Discharge with *Trichomonas vaginalis* Infection and Vaginal Bacterial Composition

Clinical characteristic	Frequency	Bacterial species isolates	Trichomoniasis		P value
			TV Positive group 10(10%)	TV Negative group 90(90%)	
Abnormal Vaginal Discharge					

Yes	57 (57%)	Lac. 55%, E. 45%,staph2%, G.3%	9 (9%)	48 (48%)	0.02642
No	43 (43%)	Lac.25%, E 10%	1 (1%)	42 (42%)	
Genital itching or irritation					
Yes	59 (59%)	Lac. 51%, E. 39%, staph13%, Can1%	4 (4%)	55 (55%)	0.19706
No	41 (41%)	Lac. 40%, E. 10%,staph epi5%,	6 (6%)	35 (35%)	
Genital sores					
Yes	22 (22%)	Lac 11%, E. 8%,staph aure.13%,	1 (1%)	21 (21%)	0.33204
No	78 (78%)	Lac 35%, staph epi.11%,	9 (9%)	69 (69%)	
Vaginal redness and swelling					
Yes	29 (29%)	Lac. 39%, E. 11%,staph9%,G3%	3 (3%)	26 (26%)	0.9442
No	71 (71%)	Lac.52%, staph epi. 30%	7 (7%)	64 (64%)	
Dyspareunia					
Yes	37 (37%)	Lac. 30%, ,staph.12%,	5 (5%)	32 (32%)	0.36812
No	63 (63%)	Lac.51%, staph epi. 13%	5(5%)	58 (58%)	

Lac: Lactobacilli **G:** Gardnerella vaginalis **E:** Escherichia coli

This study examined the prevalence of *Trichomonas vaginalis* (TV) infection and vaginal microbiota composition in women with different pregnancy histories. A statistically significant association ($P = 0.00736$) was found between a history of miscarriage and TV infection, with 8% of women experiencing miscarriage testing positive and 2% of women with normal pregnancy histories. This finding aligns with existing literature highlighting a potential link between *T. vaginalis* infection and adverse pregnancy outcomes, including miscarriage. For example, 26 found a significantly higher prevalence of *T. vaginalis* in women with a history of miscarriage than in women with successful pregnancies. The mechanisms underlying this association are not fully understood, but several hypotheses have been proposed. *T. vaginalis* infection can cause inflammation and disruption of the vaginal microenvironment, potentially increasing the risk of ascending infections and complications during pregnancy (27) as in Table 6.

Table 6. Comparison of The Vaginal Microbiota in Women with a Normal Pregnancy History Versus Miscarriage, Stratified by *Trichomonas vaginalis* Status

Pregnancy	No. of women tested for <i>T. vaginalis</i>	TV Positive group No.	Isolated microorganism		Percentage	P Value
			With T.V.	Without T.V.		
Normal history of pregnancy	46	2	<i>Lactobacilli</i>	<i>Lactobacilli</i>	2%	0.00736
			<i>E.coli</i>	<i>Staph.epidermidis</i>		
			<i>Proteus spp</i> <i>Staph. Aureus</i>	<i>G. vaginalis</i>		
Miscarriage	54	8	<i>Lactobacilli</i>	<i>Lactobacilli</i>	8%	
			<i>E. coli</i>	<i>Staph.epidermidis</i>		
			<i>S. agalactiae</i>	<i>G. vaginalis</i>		
			<i>Proteus spp</i> <i>Staph. aureus</i>	<i>Proteus spp.</i>		
Total	100	10			10%	

The highest association at a rate of 40%, and this relationship was found to be significant ($p = 0.00736$). Additionally, *Staphylococcus epidermidis* and *Lactobacillus* were associated at a rate of 30% each, followed by *Candida spp.*, *Escherichia coli*, and *Staphylococcus aureus* at a rate of 20% each. Coagulase-negative *Staphylococcus spp.*, *Prevotella spp.*, *Enterobacter spp.*, and *Streptococcus agalactiae* had the lowest association with parasite infection, each at a rate of 10%. In the *T. vaginalis*- negative group, the dominant vaginal

bacteria were *Lactobacillus* at 65.55%; this was followed by *Candida* spp. at 24.44%, *Staph. epidermidis* at 13.33%, *Gardnerella vaginalis* at 9.99%, *Staphylococcus aureus* at 7.77%, *Escherichia coli* at 6.66%, Coagulase-negative *Staphylococcus* spp. at 5.55%, *Enterobacter* spp., and *Prevotella* spp. had the lowest abundance at 3.33% and 2.22%, respectively (Table.7).

Table 7. The number and percentage of isolates of different microorganisms in the *T. vaginalis*- positive and- negative groups.

<i>Isolated Organism</i>	<i>T. vaginalis</i> Positive group		<i>T. vaginalis</i> Negative group		<i>P value</i>
	No. of isolates	Percentage	No. of isolates	Percentage	
<i>Lactobacillus</i>	3	30%	59	65.55%	0.0278
Coagulase-negative <i>Staphylococcus</i> spp.	1	10%	5	5.55%	0.57548
<i>Staphylococcus aureus</i>	2	20%	7	7.77%	0.20054
<i>Staph. epidermidis</i>	3	30%	12	13.33%	0.16152
<i>Proteus mirabilis</i>	2	20%	7	7.77%	0.20054
<i>Enterobacter</i> spp.	1	10%	3	3.33%	0.30772
<i>Escherichia coli</i>	2	20%	6	6.66%	0.14156
<i>Gardnerella vaginalis</i>	2	20%	9	9.99%	0.00736
<i>Streptococcus agalactiae</i>	1	10%	0	0%	0.00252
<i>Candida</i> spp.	2	20%	22	24.44%	0.75656

The prevalence of *T. vaginalis* infection in this study was 10%, which is within the range reported in previous studies. Specifically, the infection rate was slightly lower than the 14.73% infections in Baghdad (28) and 15.5% in Mosul (29), but higher than the 5.4% infection rate in Dohuk (30), but less than the rate reported in

Table 8. Frequency of discharge Colors *Trichomonas vaginalis* -Positive Cases

<i>Discharge color</i>	<i>PositiveTV group No.</i>	<i>Percentage</i>
Yellow to green pigment	3	30%
Frothy discharge	4	40%
Bloody discharge	2	20%
Clear discharge	1	10%
Total	10	100%

Table 8. shows three types of discharge odors: unpleasant, fishy odor, and odorless. The TV-positive group was tested for these odors, and the results were: 6 cases (60%) of an unpleasant odor, 3 cases (30%) of a fish-like odor, and 1 case (10%) of no odor in the TV-positive group. The data demonstrate that the majority (60%) of the TV- positive group experienced an unpleasant odor in their

discharge, indicating a high prevalence of unpleasant odor in this group. Additionally, 30% of the group experienced a fish-like odor, while only 10% had an odorless discharge.

Table 9. Relationship of infection with the *Trichomonas vaginalis* parasite and vaginal discharge odor.

<i>Discharge odor</i>	<i>TV Positive viewing group</i>	<i>Percentage</i>
	No.	
Unpleasant odor	6	60%
Fish-like odor	3	30%
Odorless	1	10%
Total	10	100%

The highest infection rate (20%) was observed in the 25-29 years age group. This finding agrees with previous studies (31, 32, 33), while contradicting others (34, 35, 36, 37).

There is a higher prevalence of trichomoniasis among women in rural areas compared with those in urban areas. This finding consistent with a previous study (38) but contradicts other studies (31, 36). This difference may be due to limited knowledge about the disease and its transmission routes in rural communities, as well as restricted access to healthcare services (31, 37, 38).

The data indicate that women with lower educational attainment have the highest infection rates, this result is consistent with (33, 37) but does not align with (31, 38).

Lactobacillus was the predominant species among women in the TV- negative group, accounting for 65.55%. However, the percentage of Lactobacillus dropped to 30% in the *T. vaginalis* -positive group. The difference in proportion between the two groups was statistically significant ($p =$ value of 0.0278). This result agrees with a study carried out by Torok M.R. et al. (39).

The results of this study revealed that *T. vaginalis* infection is associated with a various microorganism in the vaginal microbiota. Gardnerella vaginalis was found to have the highest association with the *T. vaginalis* parasite and changing of the period of underwear

Table 10. Association Between Underwear Changing Frequency and *Trichomonas vaginalis* Prevalence

<i>Underwear changing period</i>	<i>No. of women tested for T. vaginalis</i>	<i>TV Positive group No.</i>	<i>TV Negative group No.</i>	<i>P value</i>
Every day	61	1	60	0.00048
Every two days	22	4	18	0.14706
Every 3 days	10	3	7	0.02642
> 3 days	7	2	5	0.08914

Table 7 presents the results of different types of vaginal washes and their association with a *T. vaginalis* positivity or negativity. Sodium bicarbonate showed 2 positive and 34 negative outcomes, with a p value of 0.267, suggesting that there was no statistically significant association between the two groups. Douching had 0 positive and 23 negative outcomes, resulting in a p value of 0.06876, indicating a lack of statistical significance between the TV- positive group and the TV- negative group. The use of soap for vaginal washing showed 8 positive and 33 negative outcomes, with a statistical significance p value of 0.0083. As shown in the table, none of the participants used herbal cleaners.

Table 11. Antibiogram of the selected highly resistant isolates.

Antibiotics	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>Proteus mirabilis</i>	<i>E coli</i>	<i>K. pneumonia</i>	<i>Gardnerella vaginalis</i>	<i>Lactobacilli</i>
Ciprofloxacin	R	I	R	R	R	S	R
Trimethoprim	R	S	R	R	R	R	R
Gentamicin	S	R	R	R	I	S	S
Cefepime	R	R	R	R	R	R	R
Cefotaxime	S	R	R	R	R	S	S
Cefixime	R	R	R	R	R	R	R
Amikacin	R	I	R	R	R	S	S
Tegacyclin	R	R	R	R	R	I	R
Ceftriaxone	R	R	R	R	R	S	S
imipenem	S	S	R	S	S	R	S
Ampicillin	R	S	R	I	S	S	S
Aztreonam	R	S	S	R	S	S	S
Tobramycin	R	R	R	R	R	R	R
Amoxi-clav	R	R	R	R	R	R	R

R: resistance **S:** sensitive **I:** intermediate.

This is followed by *Staphylococcus epidermidis*. These associations were found to be statistically significant, indicating a strong relationship between the presence of *T. vaginalis* and these microorganisms. A significant relationship between the *T. vaginalis* and *Gardnerella vaginalis* bacteria was found in this study which is consistent with findings from previous studies by Al-Muqdad, S.F. et al. (40), Al-Mudhaffar, Z.M.J. (41), and Al-Murayaty, A.Y. (42). Additionally, the study reported that the association of *Candida* spp. with *T. vaginalis* was 20%, lower than the 24.44% found in the *T. vaginalis*-negative group, which is consistent with the result of other studies (43, 44). This is potentially because yeasts produce substances that inhibit the growth of the parasite. Furthermore, *Candida* spp. are known to produce substances such as organic acids and hydrogen peroxide, which create an acidic environment and have anti-parasitic properties. These substances could potentially inhibit the growth of *T. vaginalis*, leading to a lower association between the two organisms (43,45).

Conclusion

The study found that younger married women and those with lower educational levels had the highest prevalence of *T. vaginalis* infection, especially in rural areas. Also, Trichomoniasis is associated with alterations in the vaginal microbiota, particularly a decrease in the proportion of *Lactobacillus*. *T. Vaginalis* infection was strongly correlated with *Gardnerella vaginalis*, *E. coli* and *Staphylococcus epidermidis* in comparison to the *T. vaginalis*-negative group.

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