Journal of Communicable Diseases
Volume 58, Issue 3 - 2026, Pg. No. 150-157
Peer Reviewed & Open Access Journal

Research Article

Trichomonas, *Candida* and Bacterial Infection: Statistical Correlation

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DOI: https://doi.org/10.24321/0019.5138.202665

I N F O

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How to cite this article:
Kuba R H, Hasan J M, Saheb E J, Musa I S. *Trichomonas, Candida* and Bacterial Infection: Statistical Correlation. J Commun Dis. 2026;58(3):150-157.

Date of Submission: 2026-03-12
Date of Acceptance: 2026-09-29

A B S T R A C T

Background: It was only recently established that the vaginal microbiota, one of the importanthuman-microbial habitats, consists of a moderately diverse community of microbes that interact with vaginal health.

Objectives:The purpose of this study was to assess the prevalence, statistical correlations, amtimicrobial susceptibility patterns of bacterial vaginosis (BV), *Candida albicans* (*C. albicans*)., and *Trichomonas vaginalis* (*T. vaginalis*) among Iraqi women as well as their antibiotic susceptibility.

Methods: A cross-sectional study was conducted from November 2021 to October 2023, 500 vaginal swabs were collected from different clinical laboratories in Baghdad, Iraq. The ages of participants ranged from 18 to 51 years. The samples were examined usingmicroscopy, biochemical tests, cultures on appropriate media and the VITEK2 system for the identification of microorganisms. Antibiotic sensitivity tests were done usingthe Kirby-Bauer disc method for seven antibiotics (augmentin, doxycycline, ceftazidime, gentamicin, ciprofloxacin, imipenem, and levofloxacin). Statistical analysis included Chi-square test, and spearman correlation coefficients to evaluat co-infections and risk factors.

Results: Out of 500 vaginal specimens, 144 (28.2%)specimens wereidentified as an infectious agent. The highest bacterial infections 58 were recorded(22 of them non-haemolytic streptococci, 11 *Nisseria gonorrheae,* 11 *Gardnerella vaginalis* (formerly*Hemophilus vaginalis*), 10 *Escherichia coli*, and 4 were diagnosed as *Klebsiella* spp.), and *T. vaginalis* was presentin 44cases (8.8 %) while vulvovaginal candidiasis (caused by *C. albicanus*) was detected in 25 (5%). The microscopic examination showed that all 144 specimens containedpus and epithelial cells, and the statistical analysis showed significant differences of the presence of pus cells indicates the intensity of infection especially in*T.vaginalis* is more severe rather than other infections. The studt also revealed that antibiotic sensitivity was applied, making it easy to determine the best course of action for an infection.to choose the suitable treatment for infection. The microorganisms were most sensitive toimipenem and levofloxacin, followed by gentamicin,doxycycline, ceftazidime, ciprofloxacin, and augmentin.

Conclusion: The study highlightssignificant correlations and differences in the prevalence of infectious agents in vaginal infection. The higher

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percentage was for bacterial infection, followed by *T. vaginalis*, and *C. albicans*; while there was no significant difference between the species that caused the bacterial infection. Antimicrobial profilingare essential for accuurate clinical managment.

Keywords: Vaginal Microbiota, Bacterial Infection, *Candida*, *Trichomonas*, Vaginal Infection

Introduction

One particular area of the human microbiome is the vaginal microbiome. A small number of microbial species, mostly lactobacilli, that can use glycogen—which is regulated by oestrogens–define special characteristics of the vaginal environment. The vaginal environment becomes acidic due to the production of lactic acid by lactobacilli and other fermentative bacteria in conjunction with vaginal epithelial cells.Numerous infectious and non-infectious causescan lead tovaginal inflammation, also known as vaginitis. Trichomonal vaginitis (TV), vulvovaginal candidiasis (VVC), and bacterial vaginosis (BV) are the most commoncauses of infectious vaginitis.[1] At some point in their lives, 75% of women experience vaginitis. Each year, these conditionsaffectmillions of women and are strongly linked to a number of adverse outcomes. The main criteria for diagnosing vaginitis are the commonly observed clinical signs and symptoms, followed by microscopic examination of awet preparation of vaginal discharge. This examination ofthe sample is inexpensive and simple,but in reality, a variety of factors, including subjective and technical ones, can affect how accurate it is.[2] The urogenital niche is maintained and shielded from infections and their aftereffects by the vaginal bacteria. Microbiome investigations have frequently overlooked asymptomatic BV and VVC despite a great deal of research.[3]

BV is a complexcondition that is thought to be brought on by a shift in the microbial community from the normally dominant *Lactobacillus* species to a polymicrobial state, with concentrations of other aerobes and anaerobes increasing 100- and 1000-fold, respectively.[4] It is believed that women's health and illness states are correlated with the bacterial compositionof their vaginal microbiome. These compositional changes are associated withvariousphysiological states, likepregnency, menstrualcycle, and pathologicalconditionssuch as bacterial vaginosis,reflecting its dynamic nature.[5] BV, which affects 10–15% ofwomen of reproductiveage, has been linked with the infections of genital tract and adverse pregnancy complications, including chorioamnionitis, endometritis, pelvic inflammatory disease, premature rupture of membranes, intrauterine growth restriction, intrauterine foetal demise, postpartum infection, ectopic gestation, and tubal factor infertility.

Human genital and urinary tracts are parasitised by the extracellular flagellated protozoan *Trichomonas vaginalis* (*T. vaginalis*). Although *T. vaginalis* infection affects human reproductive function, it is still unclear if it canresult in infertility.[6] The most prevalent non-viral Sexually Transmitted Infections in humans is trichomoniasis, which causes a variety of symptoms in females, including increased vaginal discharge, pruritus, and perineal discomfort.[7] The World Health Organization (WHO) assessment in 2023 found that trichomoniasis affected at least 370 million people globally.[8]

According to the majority of research and review articles, *T. vaginalis* infection can cause cancer. The pathogenic processes involved areas follows: *T. vaginalis*may increases the riskof cancer by promoting an inflammatory response, modifying the physiological environment surrounding the parasite-infected-area and the signal transduction cascades, producing metabolites that cause carcinogenesis, and facilitatingother pathogenic microbial infections.[8]

Fungal vaginitis has comparable statistics. It has been observed that three-quarters of women have an asymptomatic *Candida* colony, and three out of four women have at least one episode of vaginal candidiasis. Determining a normal vaginal health environment is a significant problem, since recent research has revealed that the lower genital tract (vagina) has a surprisingly diverse microbial community. The ability to identify a balanced vaginal microbiota and connect vaginal diseases to a microbial pathogen or community structure is made possible by new techniques for researching the microbiome.[4]

Mixed vaginal infections disrupt the vaginal milieu, complicating diagnosis and treatment, and often result in recurrent infections and increased antimicrobial resistance.[9] Even though some microorganisms, like *Candida*, oftencolonise without causing any symptoms, they are truly opportunistic pathogens that can produce clinical symptoms when the host's immune system is compromised. Multiple therapies that target all pathogens causingsymptoms are necessary in cases ofmixed vaginitis, which can be classified into different categories, such as BV+VVC, BV+TV, and AV+VVC, based on their causal pathogenic processes. Clinical symptoms, pathophysiology, diagnostic standards, and treatment have not received much attention in the research of mixed vaginitis. Vaginal co-infections, characterised by clinical signs and symptoms caused by a single pathogen, and asymptomaticcolonisation by another pathogen, should be distinguished from mixed vaginitis. As a result, treatments for mixed vaginitis should only target the pathogen that producessymptoms.[10,11]

This study aims to investigate the prevalence and co-occurrence of *T. vaginalis* , *C. albicanis* and bacterial infection among Iraqi women with vaginitis and to assess

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202665

the associations between these vaginal infections and selected clinical symptoms.

Materials & Methods

Sample Collection

From November 2021 to October 2023, 500 vaginal swabs were collected from different clinical laboratories in Baghdad City, Iraq. The ages of participantsranged from 19 to 51 years.

Microscopic Examination

All the specimens were examined under a microscope for primary identification and for the examination of red blood cells, pus cells, epithelial cells and *C.albicanis.* The smears were examinedmicroscopically and diagnosed at 40x magnification. The self-collected vaginal swabs were used to extract vaginal fluids, which were then examined under a microscope to determine the presence of*T. vaginalis*,which is visible in urine sediment, cervical, and vaginal smears stained with two different staining methods, like Gram stain and Giemsa stain. When the step of staining is complete, the movement of protozoa becomes clear under the microscope.

Identification of Infection

Individuals whose reveal that they have BV they were undergo clinical and laboratory pathogenic examinations.[12,13] A thin, homogeneous discharge, an elevated vaginal pH over 4.5, the release of amines on adding 10% potassium hydroxide to vaginal fluid, and the presence of "clue" cells were the fourcriteria used to diagnose BV according to the modified Amsel's diagnostic criteria. VVC was diagnosed based ongrowing yeasts, hyphae, or pseudo-hyphae in a moist preparation (saline, 10% KOH) of vaginal discharge, or usinga culture andGram staining that yielded a positive result for a yeast species.[14] For identification of bacterial infection, culture was done on Blood and MacConkey agar and confirmed by the VITEKsystem (bioMérieux).

Antibiotic Susceptibility Test for Bacterial Infection

By using the Kirby-Bauer disk diffusion method, the antibiotic susceptibility test for bacterial infection was done.[15]The following seven antibiotic discs (HiMedia, India) at the mentioned final concentrations were used: Augmentin 30µg, doxycycline30µg, ceftazidime 30µg, gentamicin 10µg, ciprofloxacin 10µg, imipenem 10µg, and levofloxacin 5µg. These antibiotics were chosen because they are considered the most common antibiotics that have been used in the treatment of vaginal infections. The results were classified as resistant, intermediate, and sensitive, according to the inhibition zone recorded by CLSI.[16]

Ethics Approval and Consent to Participate

The Iraqi Ministry of Health (Reference: CSEC/0325/0045) and the Ethics Committee of the University of Baghdad's Department of Biology approved the study protocol. For each patient, written informed consent was obtained. The work was completed in compliance with the World Medical Association's code of ethics (Declaration of Helsinki).

Statistical Analysis

The Statistical Packages of Social Sciences (SPSS) (2019) was used to determine the effects of various research parameters. Percentages were comparedusing the chi-square test, with significance set at 0.05 and 0.01.[17]

Results

Out of 500 vaginal specimens, 144 (28.2%) of the specimens showed signs of infection. Among them, 44 specimens contained *T. vaginalis,* 17 showed *C. albicans*, 58 were found to bebacterial infections (22 of them non-haemolytic streptococci, 11 *Gardnerella vaginalis*, 11 *Neisseria gonorrheae*,10 *Escherichia coli*, and 4 were diagnosed as *Klebsiella* spp.), and 25 specimens containedother *Candida* spp. The microscopic examination showedthat all 144 (28.2%) specimens contained pus and epithelial cells.

Table 1.Percentage Positivity of Infectious Agents

Infectious Agents	No. of Specimens	Percentage from 144	Percentage among Total Specimens	p Value
T. vaginalis	44	30.55	8.8	0.0001**
Other Candida spp.	25	36.00	5.0	0.0001**
C. albicans	17	24.48	3.4	0.0001**
p Value	-	0.0380*	0.1740 NS	-
Bacterial species	58	40.27	11.6	0.0001**
Non-haemolytic streptococci	22	15.27	4.4	0.0084**
Gardnerella vaginalis	11	7.63	2.2	0.2190 NS
N. gonorrhoeae	11	7.63	2.2	0.2190 NS
E. coli	10	6.94	2.0	0.2550 NS

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202665

Klebsiella spp.	4	2.77	0.8	0.6730 NS
p Value	-	0.0091**	0.3610 NS	-

** Highly significant (p ≤0.01)
NS: Non-significant

Table 2.Association between Age Rangeand Type of Infection

Infectious Agent	Age Range (Years)				Total	p Value
	15–25 Younger women	25–35 Reproductive age	35–45 Late reproductive age	45–55 Menopausal transition age		
T. vaginalis	7	15	11	11	44	0.0368*
C. albicans	6	3	7	1	17	0.0094**
Other Candida spp.	8	3	7	7	25	0.0415*
p value	0.7520 NS	0.0037**	0.2160 NS	0.0062**	-	-
Bacterial species						
Non-haemolytic streptococci	0	1	10	11	22	0.0002**
Gardnerella vaginalis	0	1	5	5	11	0.0274*
N. gonorrhoeae	0	1	7	3	11	0.0084**
E. coli	0	1	7	2	10	0.0087**
Klebsiella spp.	0	0	0	4	4	0.0214*
Total 13 22 47 37					144	-
p value	0.0000 NS	0.8720 NS	0.0062**	0.3950 NS	-	-

* Significant (p ≤0.05), ** Highly significant (p ≤0.01)
NS: Non-significant

Table 3. Antibiotic Resistance Profilesof Bacterial Isolates for SevenAntibiotics by Kirby-Bauer Method according to CLSI (2023)

Antibiotic	Resistance n (%)	Moderate n (%)	Sensitive n (%)	p Value
AMC	39(70.90)	1(1.80)	15(27.27)	0.0001**
DO	25(45.45)	0(0.00)	30(54.54)	0.0001**
CAZ	15(27.27)	16(29.09)	24(43.63)	0.0259*
Gen	0(0.00)	5(9.09)	50(90.90)	0.0001**
CIP	0(0.00)	39(70.90)	16(29.09)	0.0001**
IPM	0(0.00)	0(0.00)	55(100.00)	0.0001**
LE	0(0.00)	0(0.00)	55(100.00)	0.0001**
p value	0.0001**	0.0001**	0.0001**	-

* Significant (p ≤0.05),** Highly significant (p ≤0.01)
R: Resistance, M: Moderate, S: Sensitive

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202665

Table 4.Intensity ofSpecimens under Study

Intensity Score	Candida (N = 25) n (%)	T. vaginalis (N = 44) n (%)	p Value
0 (nil)	0 (0.00)	0 (0.00)	NS
+	3 (0.00)	0 (0.00)	0.0490*
++	16 (0.00)	1	0.0001**
+++	0 (0.00)	11	0.0006**
++++	6 (0.00)	7	0.902 NS
+++++	0 (0.00)	16	0.0001**
Too much to count	0 (0.00)	9	0.0025**
p value	0.0001**	0.0001**	-

* Significant (p ≤0.05), ** Highly significant (p ≤0.01)
NS: Non-significant

Table 5.Spearman correlation between Age and infectious Agents in the studied samples

Infectious Agent and Bacterial species	Spearman correlation-r with Age	P-value
T. vaginalis	0.35 *	0.0497
C. albicans	-0.41 *	0.0482
Other *Candida* spp.	0.06 NS	0.7685
Non-haemolytic streptococci	0.73 **	0.0001
Gardnerella vaginalis	0.68 **	0.0004
N. gonorrhoeae	0.52 **	0.0056
E. coli	0.67 **	0.0004
Klebsiella spp.	0.41 *	0.0483
Total	0.57 **	0.0045
* (P≤0.05), ** (P≤0.01).		

* Significant (p ≤0.05), ** Highly significant (p ≤0.01)
NS: Non-significant

Discussion

Mixed vaginitis occurs when many pathogenic processes are present at the same time and are mediated by at least two different kinds of vaginal pathogens. The most common manifestation of mixed vaginitis among the different kinds is the concurrent occurrence of BV andVVC. Worldwide, mixed vaginitis has an impact on women's health at every age. The clinical signs, pathophysiology, diagnostic standards, and treatment of mixed vaginitis, however, have not received much attention in research.

These results revealed that there were highly significant differences in the prevalence rates of vaginal infection among the causative infectious agents. The highestprevalence percentage was for bacterial infection (11.6%), followed by *T. vaginalis* (8.8%),*Candida* (5.0%), and *C. albicans* (3.4%). Nosignificant difference was observed amongthe bacterial species that caused bacterial infection. According to earlier data, the prevalence of trichomoniasis was 5.3% in women and 0.6% in men worldwide, with a rising trend,[18] 1.8% and 0.5% in women and men, respectively, in the United States,[19] and 1.7% in France.[20]

A highly significant association was observed between the age range of patients and the kinds of vaginal infection, with the age range of 35–55 years showing the greatestpercentage of vaginal infection.

The findings of the study byXiao et al. provide insightsinto improving the cure rate and reducing recurrence.[21] The study looked at the diversity, composition, and other characteristics of the vaginal microbiome in patients with mixed vaginitis (BV+VVC),both before and after treatment. Itshowed that the combined medication therapy proved successful in treating mixed vaginitis, and the results of the treatment suggested that better BV treatment might be more advantageous due to the increased difficulty of treating BV.According to the findings of a study conducted by Anh*et al*., Compared to many other regions of the world, Vietnam has a higherprevalence of VVC among symptomatic non-pregnant women of reproductive age. One noteworthy

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202665

aspect was the high prevalence of non-*albicansCandida* species, which were frequently more resistant to antifungal medications. Antifungal agent resistance was minimal among vaginal *C. albicans* isolates. routine screening and treatment should be done in order to monitor changes in species distribution and susceptibility to antifungals.[22]

These results of the antibiotic resistance profile showed that the bacteria were most sensitive to imipenem and levofloxacin, followed by gentamicin,doxycycline, ceftazidime, ciprofloxacin, and augmentin.

In a 2016 local study, Saheb*etal.* found that 525 (85.5%) of 614 females had positive *T. vaginalis* screening results. However, 89 of the 614 females tested negative for *T. vaginalis* and instead developed another vaginal infection.[23] Seven (1.14%) of the tested females had *Candida* infection, whereas 82 (13.36%) had bacterial infections, including *Gardnerella vaginalis*, *E. coli*, and *Pseudomonas* spp. Furthermore, the majority of infected females were in the age category of over 30 years. For *Trichomonas* infections, 122 patients (85.31%) had four pus cells per field of microscope; for bacterial infections, 29 patients (69.05%) had two pus cells per field of microscope; and for *Candida* infections, 2 patients (33.33%) had two statistically significant pus cells per field of microscope. The study's findings showed that *T. vaginalis* is endemic in Baghdad and that its intensity rate is higher in females than in those infected with bacteria or *Candida*. The spread of this parasite could be managed by preventing *T. vaginalis* infections.

According to a[24],more than one-third of non-pregnant reproductive-aged women seeking Primary Health Care in Sana'a had single or mixed infections with BV, VVC, or TV, that sought to ascertain the prevalence and risk factors associated with BV, VVC, and TV among these women. While the prevalence of VVC wassignificantly higheramong women with polygamous spouses, BV, the most common cause of vaginitis, wassignificantly associatedwith being younger than 25 years and using Intrauterine Contraceptive Devices. It is advised that polygamous couples receive health education, Intrauterine Contraceptive Devices users regularly undergo screening for BV, and women arescreened for vaginitis prior to treatment.

Zhang*et al.*'s study supported the idea that *T. vaginalis* infection and infertility are related, and that the infection itself can cause infertility.[6] Their research lays the groundwork for future research into the pathophysiology of *T. vaginalis* infection. Additionally, Zhang*et al*. verified that *T. vaginalis* infection was may be linked to cancer of the reproductive system and offered several potential research avenues to elucidate the carcinogenic mechanisms of *T. vaginalis* infection.[8]

The spearman correlation analysis demonstrated significant associations between age and the establishment of several infectious agents. The significant positive correlation was revealed between age and the protozoan parasite *T. vaginalis* (r = 0.35, p = 0.0497), that means there is a tendency for the occurrence of this infection that increase with age, while *C. albicans* infections showed a significant negative correlation with age (r = -0.41, p = 0.0482), that means candiasis decrease when the is increases. No significant correlation was detected between age and other *Candida* spp. (r = 0.06, p = 0.7685). In contrast, bacterial isolates, revealed that there are significant positive correlations for non-haemolytic *streptococci* (r = 0.73, p = 0.0001). *Gardnerella vaginalis* (r = 0.68, p = 0.0004), *E. coli* (r = 0.67, p = 0.0004), N. gonorrhoeae ((r = 0.52, p = 0.0056), and *Klebsiella* spp (r = 0.41, p = 0.0483). The strongest of the correlation was observed for non-haemolytic *streptococci*, followed by *Gardnerella vaginalis* and *E. coli*. The total establishment of infectious agents was positively correlated with age (r = 0.57, p = 0.0045), that means older age women are associated with ahigher frequency of pathogenic agents in studied population.

Conclusion

This study concluded that there were highly significant differences in the prevalence of infectious agents in vaginal infection. The higher percentage was for bacterial infection, followed by *T. vaginalis*, *Candida*, and *C. albicans*; while there was no significant difference between the species that caused the bacterial infection. The age range of the patients showed a highly significant association with the kinds of vaginal infection,revealing that the age rangeof 35–55 years had the greatestpercentage of vaginal infection with different infectious agents. The antibiotic resistance profile showed that the bacteria were most sensitive to imipenem and levofloxacin, followed by gentamicin,doxycycline, ceftazidime, ciprofloxacin, and augmentin and the severityof infection was high with the infection with *T. vaginalis* than other types of infection. The total establishment of infectious agents was positively correlated with age (r = 0.57, p = 0.0045), that means older age women are associated with ahigher frequency of pathogenic agents in studied population. More studies are recommended todetect vaginal microorganisms and to investigate theirpathogenicity for treatment and therapy.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions

RHK and JMH contributed to the idea and design of the research. All authors were involved in the study

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202665

methodology. RHK and JMH contributed to the data collection and analysis for the study. JMH was involved in drafting the manuscript. All authors have read and agreed to the published version of the final manuscript.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: The authors declared that no AI or AI-Assisted Technologies were used in the Writing Process of this manuscript.

Conflict of Interest: None

Source of Funding: None

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ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202665