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RESEARCH

Unveiling shifts in cervical microbiota composition among Colombian women in the presence of *Trichomonas vaginalis*: a longitudinal study

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Abstract

Trichomonas vaginalis is a flagellated protozoan affecting human reproductive health. Although bacterial coinfections have been associated with *T. vaginalis* infection outcomes, pertinent data are scarce. This study was aimed at assessing vaginal bacterial community changes regarding *T. vaginalis* outcomes (infection, clearance, persistence) during patients' follow-up visits. This was a 1-year follow-up study involving Colombian women who provided two cytological scrapings from the cervix (one at baseline and another at follow-up), having an average 12 ± 1 month follow-up interval. *T. vaginalis* was detected by conventional PCR (Tvk3/7 and BTU9/2 primers). The Illumina Novaseq PE250-platform was used for assessing microbiota composition. This study involved 66 women (132 samples); 68.2% ($n = 45$) tested negative for *T. vaginalis* at baseline while 31.8% ($n = 21$) were initially diagnosed with *T. vaginalis* infection (99,304 amplicon sequence variants identified/categorised into 62 phyla and 1908 genera). Women who cleared *T. vaginalis* ($n = 10$) displayed increased microbial richness, while those having persistence ($n = 11$) had higher microbial diversity. Significant changes were observed regarding genus relative abundance: *Lactobacillus* abundance increased in the clearance group ($p = 0.002$), *Prevotella* in the persistence group ($p = 0.045$) and *Gardnerella*, *Megasphaera* and *Sneathia* in the group having acquired *T. vaginalis* infection ($p = 0.045$). Regarding relative abundance, logistic regression analysis revealed a positive trend concerning *Sneathia* increase (adjusted OR 2.23) and reduced *Lactobacillus* abundance (adjusted OR 0.39) in women with *T. vaginalis* persistence. DESeq analysis revealed a substantial decrease in the *Ornithinimicrobium* genus among women without infection during follow-up, while *Ruminococcus* increased in women having various *T. vaginalis* outcomes. The results suggested that specific bacterial genera present during *T. vaginalis* infection may influence its clearance, having potential implications for improving diagnostic and therapeutic strategies for enhancing women's reproductive health.

Keywords *Trichomonas vaginalis* · Follow-up · Epidemiology · Next-generation sequencing · Cervical microbiota

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Introduction

Trichomonas vaginalis ranks as the most common sexually transmitted infection (STI) having a parasitic origin. It has been estimated that more than two million women worldwide are infected, with approximately 50% being asymptomatic (Lewis et al. 2021; WHO 2024). This prevalence is significant when compared to other non-viral STIs, such as syphilis or gonorrhoea, highlighting the underdiagnosis and limited awareness regarding this infection (WHO 2024). *T. vaginalis* infection is not subject to mandatory reporting nor surveyed in public health programmes; it is currently considered a neglected disease (Menezes et al. 2016).

T. vaginalis infection has been linked to adverse birth outcomes, infertility and its increased susceptibility to other STIs, such as human immunodeficiency virus (HIV), the persistence of high-risk human papillomavirus (HPV) types and the subsequent development of cervical cancer (Kissinger et al. 2022; Mielczarek and Blaszkowska 2016). Older women, Afro-descendants, low socioeconomic status and a greater amount of sexual partners have been identified as risk factors for infection (Tompkins et al. 2020). The potential contribution of the cervical microbiota to a synergistic effect involving *T. vaginalis* infection by modulating the microenvironment has been highlighted recently (Kelvin Stefan et al. 2023; Margarita et al. 2020). Such modulation promotes the use of vaginal substrates (i.e. free iron) for enhancing *T. vaginalis* virulence, ultimately promoting its persistence in a host and causing ongoing and progressive damage to cervical architecture (Leitsch 2021).

The symbiotic relationship between *Mycoplasma* hominis and *T. vaginalis* is the most frequently described in the literature (Fichorova et al. 2017). Such relationship produces changes in the cervical environment, such as arginine metabolism by *M. hominis* facilitating the *T. vaginalis* lifecycle by increasing ATP availability. *T. vaginalis* proliferation and persistence produce metabolites such as indole (released by the parasite), contributing to anaerobic bacteria overgrowth; such overgrowth contributes to malignant transformations in the cervical epithelium (Dessi et al. 2019).

Although this relationship is significant, further information is needed to better understand the clinical progression of *T. vaginalis* infection and its interaction with other microorganisms in the cervical environment. Previous studies have suggested that microbial communities play a crucial role in parasite colonisation and pathophysiology (Fichorova et al. 2017). Given the importance of *T. vaginalis* in altering vaginal microbial ecology and limited knowledge regarding which bacterial species are involved in *T. vaginalis* persistence, this study has investigated the relationship between *T. vaginalis* and cervical microbiota through a retrospective cohort study involving Colombian women.

Materials and methods

This study arose from a bidirectional cohort recruited from April 2007 to March 2010 to investigate the natural history of HPV infection (Soto-De León et al. 2014). Current analysis considered two time-points: baseline and a 12-month follow-up (± 1 month). Sixty-six women were selected, based on the availability of samples from both time-points. Participants gave their written informed consent, completed detailed questionnaires on sociodemographic, reproductive and sexual behaviour and received medical care at Engativá Level II Hospital in Bogotá, Colombia. The study was approved by the aforementioned hospital's institutional ethics committee (approval number CEHE-009) and conducted in accordance with the Declaration of Helsinki and Colombian Ministry of Health guidelines.

Cervical samples were collected during cytology procedures and preserved at 4 °C in a 95% ethanol-based transport medium (Sigma-Aldrich, St. Louis, MO, USA). A QuickExtract solution kit (Epicentre, Madison, WI, USA) was used for extracting genomic DNA which was then stored at − 30 °C. HPV detection was performed using three generic primer sets (GP5 + /GP6 + , MY09/MY11 and pU1M/2R), as described in previous studies (Soto-De León et al. 2014). *T. vaginalis* was detected via conventional PCR using Tvk 3/7 and BTU 9/2primer sets, as previously described (Hernandez-Buelvas et al. 2021). These methodologies ensured molecular analysis reliability and provided a robust dataset for evaluating changes between both time-points. Additional cohort information and detailed protocols are available in the cited publications.

T. vaginalis infection outcomes were assessed, i.e. absence, acquisition, clearance and persistence. Amplicon-based sequencing of the 16S rRNA hypervariable region V4 was used for analysing bacterial communities from samples collected during both visits. The Illumina Novaseq PE250 platform was used for sequencing; this gave 250 bp raw reads, having a minimum expected depth of 100,000 reads per sample. The datasets used in this study are available online in the European Nucleotide Archive (ENA) under EMBL-EBI project accession PRJEB55377.

QIIME2 software (version 2019.7) was used for demultiplexing and removing barcodes and primers from raw reads (Caporaso et al.,2010). A Phred score of 30 or higher was considered as a quality filter; forward; reverse reads were then merged. The central sample inference algorithm gave product amplicon sequence variants (ASV), discarding additional sequences having 100% nucleotide identity. Chimeras were then removed from ASV. R studio (version 4.3.2) DADA2 was used for such procedures and taxonomic assignment by comparing ASV sequences against

the 16S rRNA SILVA v132.16 s database, considering bootstrap 50 (Callahan et al. 2016).

Taxonomic assignment was analysed for determining relative abundance (concerning total reads obtained per sample) for different taxonomic levels. A graph of predominant genera regarding *T. vaginalis* outcome was constructed, followed by alpha diversity analysis (Abundance-based coverage estimator (ACE), Shannon and Simpson) and beta diversity analysis, using non-metric multidimensional scaling (NMDS) and principal coordinates analysis (PCoA), both based on Bray–Curtis similarity index matrices. Permutational analysis of variance (PERMANOVA) distance matrix variation within a group was used for evaluating statistical differences concerning sample clustering re *T. vaginalis* outcome.

A Kruskal–Wallis test was used for evaluating statistical differences, followed by a post-hoc Dunn test with a Benjamini–Hochberg correction (false discovery rate) (Wickham 2016). RStudio's phyloseq package was used for all analyses; ggplot (McMurdie and Holmes 2013) was then used for visualising the results.

The DESeq2 package was used for assessing significant differences regarding the abundance of bacteria in each group; the Wald test was used for detecting differences regarding the abundance of predominant genera by *T. vaginalis* infection outcome. A < 0.01 *p*-value (adjusted by Benjamini–Hochberg correction) was considered statistically significant.

Associations between bacterial genus and *T. vaginalis* outcome (without infection, acquired infection, clearance and persistence) were assessed using conditional logistic regression. Adjusted odds ratios (ORs), along with their 95% confidence intervals (CIs), were estimated in the model.

Age, age at first intercourse, number of pregnancies, the number of lifetime sexual partners and contraceptive methods were included as covariates. STATA14 software was used for all two-tailed statistical tests, and $p < 0.05$ values were considered statistically significant.

Results

One hundred and thirty-two samples taken from 66 women were analysed at two follow-up points over a year-long study. The study population's sociodemographic characteristics have been published previously (Camargo et al.,2022). The results showed that 68.2% ($n = 45$) of the women began the study without *T. vaginalis* infection (Supplementary data, Fig. S1); 37.8% ($n = 17$) of them acquired the infection during the 1-year study period while 31.8% ($n = 21$) of the women had *T. vaginalis* infection at baseline, 47.6% of them clearing the infection as time elapsed (Supplementary data, Fig. S1).

The samples that underwent 16S rRNA (V4-region) amplicon-based sequencing were quality-controlled and clustered into 99,304 ASVs. These were assigned to 62 phyla and 1908 bacterial genera. Other information related to this retrospective cohort's cervical microbiota composition has been published previously (Camargo et al.2022).

Richness and abundance medians were analysed concerning *T. vaginalis* infection outcomes. The ACE richness estimator was used for calculating species richness, while Shannon–Weaver and Simpson indices were used for estimating abundance. There was greater richness in women who cleared compared to those having persistence; by contrast, the diversity index showed greater diversity in the persistence group (Fig. 1). However, no significant differences between medians were observed in diversity analysis (richness and abundance p-value > 0.05). Principal coordinates analysis (PCoA) and PERMANOVA analysis did not indicate apparent clustering and/or significant differences in the groups analysed.

Descriptive results showed that the bacterial communities' composition varied between the two time-points evaluated (baseline and 12-month follow-up), significant changes occurring regarding certain genera's relative abundance: *Lactobacillus* spp. in the clearance group ($p = 0.002$), *Prevotella* spp. in the persistence group ($p = 0.045$) and *Gardnerella* spp., *Megasphaera* spp. and *Sneathia* spp. in the *T. vaginalis* infection acquisition outcome group ($p = 0.045$ for all species) (Fig. 2). Logistic regression analysis revealed a positive trend for relative abundance, increasing for *Sneathia* spp. in women having *T. vaginalis* persistence (2.23 adjusted OR, 1.05–4.72 95% CI, $p = 0.024$); by contrast, a reduction in relative abundance was observed for *Lactobacillus* spp. (0.39 adjusted OR, 0.05–0.72 95% CI, $p = 0.032$).

DESeq was used for identifying the genera differentially present concerning *T. vaginalis* outcome (Fig. 3). Two genera had significant results; *Ornithinimicrobium* spp. decreased in women without infection during follow-up compared to the other events evaluated (acquired infection, clearance and persistence) while *Ruminococcus* spp. had a significant increase regarding all outcomes (without infection, acquired infection and persistence) compared to clearance (Fig. 3).

Discussion

The current study has explored the relationship between *T. vaginalis* infection outcome and cervical microbiota composition, providing fresh insights into the dynamic interactions between these infections and cervical microbiota as time elapsed. Such longitudinal analysis has highlighted significant shifts in bacterial diversity and abundance, thereby contributing to our understanding of how *T. vaginalis*

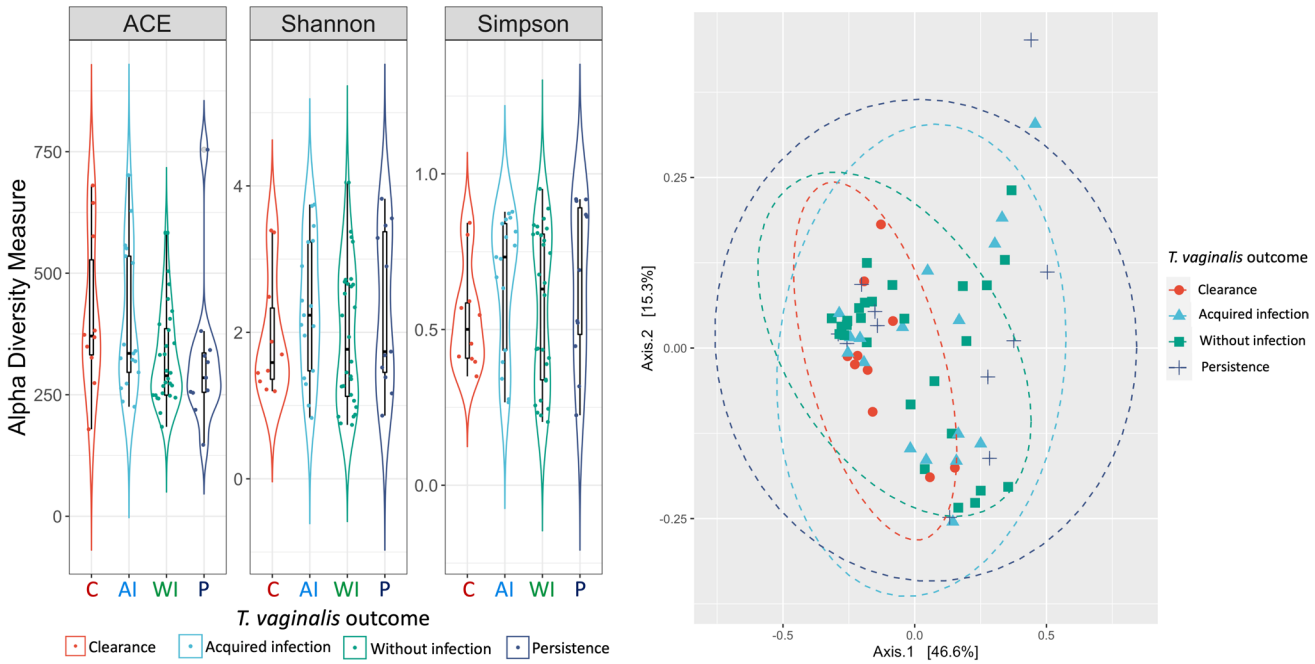

Fig. 1 Alpha and beta diversity regarding *T. vaginalis* outcome. A Kruskal–Wallis test was used for evaluating significant differences between the study groups

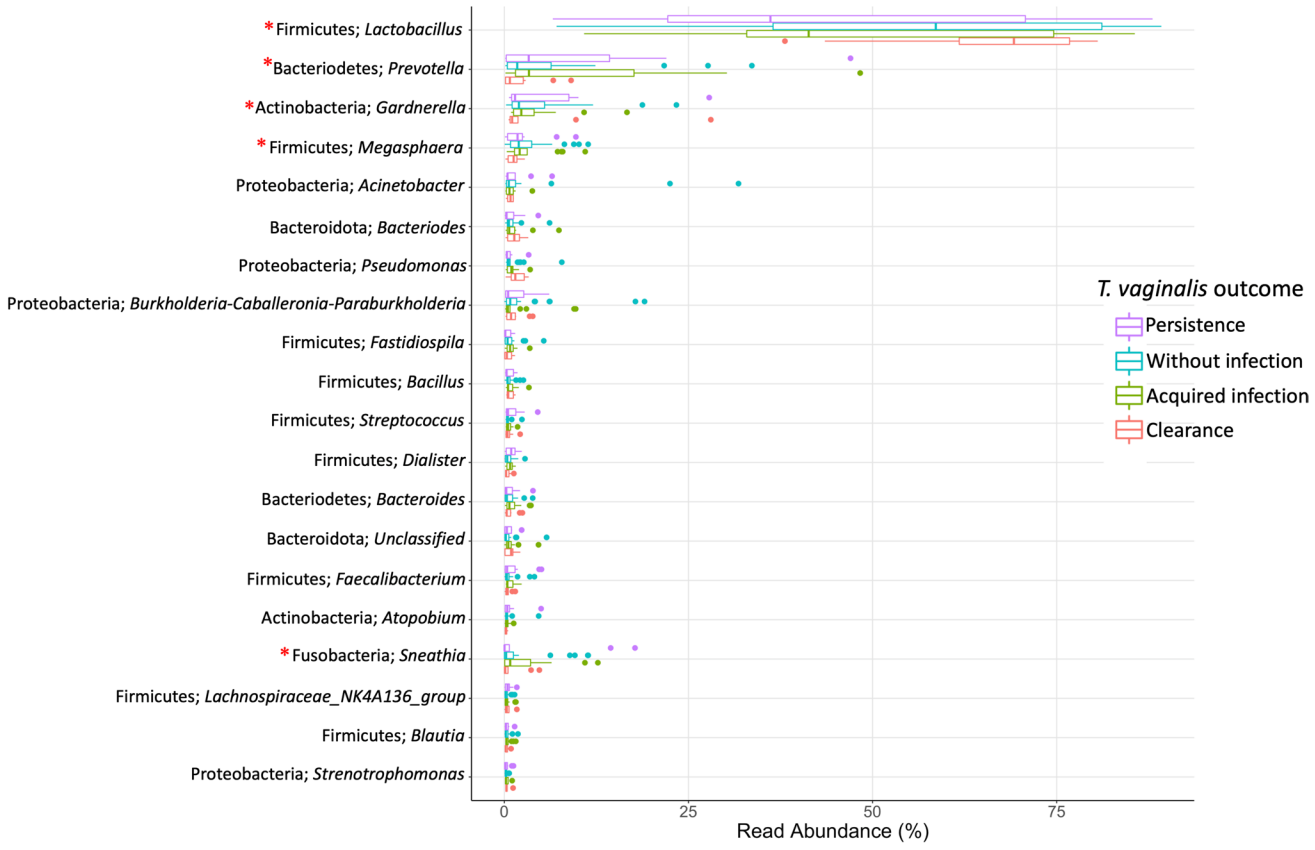

Fig. 2 Boxplot showing the differences between *T. vaginalis* outcome by each genus' relative abundance, along with their phyla. * Statistically significant differences regarding T. vaginalis outcome

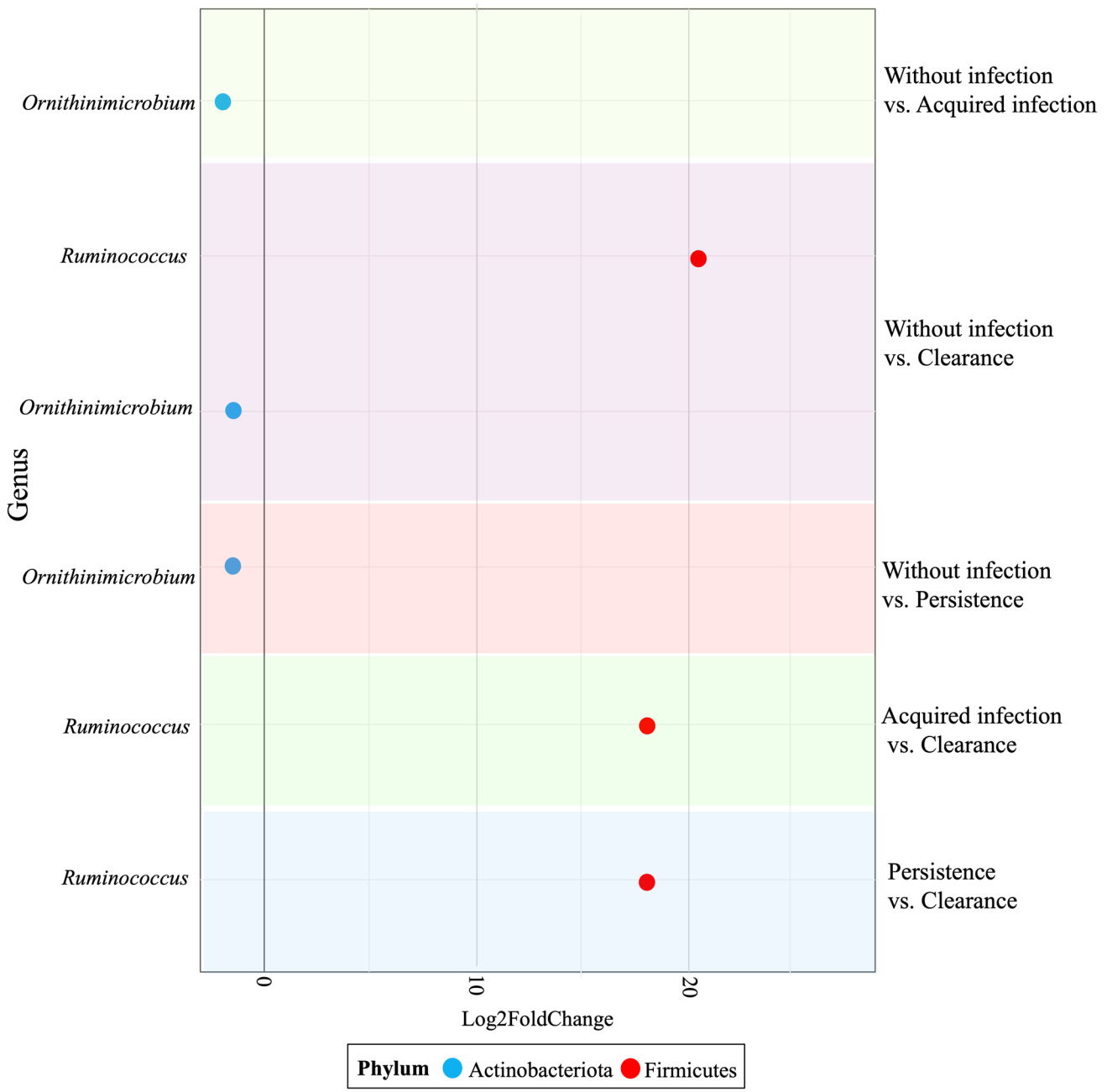

Fig. 3 DESeq2 analysis was used to identify differentially abundant genera concerning *T. vaginalis* outcome

persistence and clearance may be influenced by the cervical microenvironment. A key finding was the increased diversity observed in women having *T. vaginalis* persistence along with distinct bacterial genera associated with specific infection outcomes. These findings offer a novel perspective concerning microbiota's role in influencing *T. vaginalis* infection dynamics.

Previous studies, including our earlier research on high-risk HPV infection (Camargo et al.2022), have shown that viral loads are linked to changes in microbiota diversity. Women having higher viral loads have had a reduction in *Lactobacillus* spp. and greater bacterial diversity, thereby aligning with the dysbiotic state. The current study has built on such a foundation by extending research to *T. vaginalis* infection, bridging gaps in understanding the microbial dynamics involved in parasitic and viral infection. This represents a critical contribution as similar trends have been observed in other studies, even if not statistically significant, thus underlining the importance of such microbial shifts in infection persistence and clearance.

One of the study's key findings has been the association between *T. vaginalis* persistence and relative *Sneathia* spp. abundance (Fig. 2), such genus having previously been implicated in adverse reproductive outcomes. Reduced *Lactobacillus* spp. abundance in women having *T. vaginalis* persistence (Fig. 2) agreed with prior studies highlighting these bacteria's protective role in maintaining vaginal homeostasis. *Lactobacillus* spp. produce metabolites such as lactic acid, hydrogen peroxide and bacteriocins which reduce oxidative stress and acidify the vaginal environment (Chen et al. 2020; Fichorova et al. 2017; Mitra et al. 2015). Reduced *Lactobacillus* spp. presence may thus compromise such protective functions, thereby facilitating parasite persistence and recurrent infection. These observations underscore the importance of bacterial community structure in modulating infection outcomes and suggest potential pathways by which *T. vaginalis* persistence may become facilitated.

Interestingly, *Ruminococcus* spp. were differentially observed in women having infection clearance and *Ornithinimicrobium* spp. in those without infection (Fig. 3). While *Ruminococcus* spp. is primarily described as a gut commensal, its role in cervical health remains unclear. Some species have been implicated in proinflammatory responses and endometritis (Wang et al. 2021), suggesting possible crosstalk between gut and cervical microbiota (Amabebe and Anumba 2020; Vega et al. 2022). *Ornithinimicrobium* spp., described in intestinal environments (Zhao et al. 2022), has been linked to amino acid metabolism and HPV cases involving multiple viral types (Zeng et al. 2022) as well as having an impact on potential fertility (Yagisawa et al.,2023). Further research is needed to determine whether these genera directly interact with *T. vaginalis* or whether their association arises from broader environmental shifts in the microbiome.

This study has highlighted the cervical microbiota's potential impact on *T. vaginalis* infection dynamics and treatment outcomes (Hirt and Sherrard 2015; Kissinger et al. 2010). Although associations with specific bacterial genera were observed, the study's descriptive nature, its small sample size and limited follow-up time require cautious interpretation. Nonetheless, *Prevotella* spp., *Sneathia* spp. and *Megasphaera* spp. coexistence, along with a reduction in *Lactobacillus* spp., suggests that a polymicrobial environment could facilitate *T. vaginalis* colonisation and persistence. Such findings agree with previous research indicating that microbial interactions (such as cytoadherence and cytoskeletal alterations) contribute to parasite colonisation by disrupting the cervical epithelial barrier (Chen et al. 2020; Fichorova et al. 2017; Mitra et al. 2015). Future studies involving larger cohorts and extended follow-up are needed to validate such observations and elucidate these interactions' underlying mechanisms.

This study's implications extend beyond microbiota characterisation, providing practical insights for managing infections like *T. vaginalis.* The observed microbial shifts could provide data concerning strategies for early detection, targeted interventions and microbiome-based therapies aimed at restoring cervical health. Additionally, a broader ecological perspective on microbial interactions may improve our understanding of pathogen dynamics and their impact on reproductive health.

This study has thus offered novel contributions to microbial ecology and parasitology by exploring the relationships between *T. vaginalis* infection outcomes and cervical microbiota composition. While our findings highlight significant trends, they also underscore the need for further research to validate such associations and explore their clinical implications. Understanding the coexistence of microorganisms and their impact on infection dynamics is essential for developing effective strategies for addressing *T. vaginalis* and other reproductive health challenges.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-025-08482-4.

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Author contribution MC, MM, JDR and MAP conceived, designed, and supervised the study. LHB, LP and IMBM collected data and detected pathogens. MC, LV, and MM data curation and conducted the analyses. MC, LV, MM, LHB, LP, JDR and MAP writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability Data will be made available on request.

Declarations

Ethics approval and consent to participate The study was supervised and approved by the Engativá Level II Hospital's ethics committee (approval no. CEHE-009), and all protocols were carried out following the Declaration of Helsinki and the Colombian Ministry of Health and Social Protection guidelines.

Competing interests The authors declare no competing interests.

References

Amabebe E, Anumba DOC (2020) Female gut and genital tract microbiota-induced crosstalk and differential effects of short-chain fatty acids on immune sequelae. Front Immunol 11:2184

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13(7):581–583. https://www.ncbi.nlm.nih.gov/pubmed/27214047

Camargo M et al (2022) Changes in the cervical microbiota of women with different high-risk human papillomavirus loads. Viruses 14(12). https://doi.org/10.3390/v14122674

Caporaso JG et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7(5):335–336. https://www.ncbi.nlm.nih.gov/pubmed/20383131

Chen Y et al (2020) Human papillomavirus infection and cervical intraepithelial neoplasia progression are associated with increased vaginal microbiome diversity in a Chinese cohort. BMC Infect Dis 20(1):629. https://doi.org/10.1186/s12879-020-05324-9

Dessi D, Margarita V, Cocco AR, Marongiu A, Fiori PL, Rappelli P (2019) Trichomonas vaginalis and mycoplasma hominis: new tales of two old friends. Parasitology 146(9):1150–1155. https://www.ncbi.nlm.nih.gov/pubmed/30616707

Fichorova R, Fraga J, Rappelli P, Fiori PL (2017) Trichomonas vaginalis infection in symbiosis with trichomonasvirus and mycoplasma. Res Microbiol 168(9–10):882–891

Hernandez-Buelvas L, Camargo M, Sanchez R, Patarroyo ME, Patarroyo MA (2021) Trichomonas vaginalis follow-up and persistence in Colombian women. Sci Rep 11(1):22597. https://www.ncbi.nlm.nih.gov/pubmed/34799668

Hirt RP, Sherrard J (2015) Trichomonas vaginalis origins, molecular pathobiology and clinical considerations. Curr Opin Infect Dis 28(1):72–79

Kelvin Stefan O, Wenyu L, Binhua D, Pengming S (2023) Trichomonas vaginalis and human papillomavirus: association with the microbiota and burden on the cervix. Gynecology and Obstetrics Clinical Medicine 3(4):null. https://gocmsite-bmj.vercel.app/content/3/4/207

Kissinger PJ et al (2022) 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 Infectious Diseases 74(Supplement_2):S152–S161. https://doi.org/10.1093/cid/ciac030

Kissinger P et al (2010) A randomized treatment trial: single versus 7-day dose of metronidazole for the treatment of trichomonas vaginalis among HIV-infected women. J Acquir Immune Defic Syndr 55(5):565–571

Leitsch D (2021) Recent advances in the molecular biology of the protist parasite Trichomonas vaginalis. Fac Rev 10:26. https://www.ncbi.nlm.nih.gov/pubmed/33718943

Lewis FMT, Spicknall IH, Flagg EW, Papp JR, Kreisel KM (2021) Incidence and prevalence of Trichomonas vaginalis infection among persons aged 15 to 59 years: United States, 2018. Sex Transm Dis 48(4):232–237. https://www.ncbi.nlm.nih.gov/pubmed/33492095

Margarita V, Fiori PL, Rappelli P (2020) Impact of symbiosis between Trichomonas vaginalis and Mycoplasma hominis on vaginal dysbiosis: a mini review. Front Cell Infect Microbiol 10. https://www.frontiersin.org/journals/cellular-and-infection-microbiology/articles/10.3389/fcimb.2020.00179

McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS one 8(4):e61217. http://www.ncbi.nlm.nih.gov/pubmed/23630581

Menezes CB, Frasson AP, Tasca T (2016) Trichomoniasis - are we giving the deserved attention to the most common non-viral sexually transmitted disease worldwide? Microb Cell 3(9):404–419. https://www.ncbi.nlm.nih.gov/pubmed/28357378

Mielczarek E, Blaszkowska J (2016) Trichomonas vaginalis: pathogenicity and potential role in human reproductive failure. Infection 44(4):447–458. https://doi.org/10.1007/s15010-015-0860-0

Mitra A et al (2015) Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. Sci Rep 5:16865

Soto-De León SC et al (2014) Persistence, clearance and reinfection regarding six high risk human papillomavirus types in Colombian women: a follow-up study. BMC Infect Dis 14:395

Tompkins EL, Beltran TA, Gelner EJ, Farmer AR (2020) Prevalence and risk factors for Trichomonas vaginalis infection among adults in the U.S., 2013–2014. PLoS One 15(6):e0234704. https://www.ncbi.nlm.nih.gov/pubmed/32544192

Vega L, Bohórquez L, Ramírez JD, Muñoz M (2022) Do we need to change our perspective about gut biomarkers? A public data mining approach to identify differentially abundant bacteria in intestinal inflammatory diseases. Front Cell Infect Microbiol 12:918237

Wang J et al (2021) Translocation of vaginal microbiota is involved in impairment and protection of uterine health. Nat Commun 12(1):4191. https://doi.org/10.1038/s41467-021-24516-8

WHO (2024) Sexually transmitted infections (STIs). https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-%28stis%29. Accessed Feb 21 2025

Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer

Yagisawa T et al (2023) Metataxonomic analysis of the uterine microbiota associated with low fertility in dairy cows using endometrial tissues prior to first artificial insemination. Microbiol Spect e0476422. https://doi.org/10.1128/spectrum.04764-22

Zeng M et al (2022) Roles of vaginal flora in human papillomavirus infection, virus persistence and clearance. Front Cell Infect Microbiol 12:1036869

Zhao H, Yuan L, Zhu D, Sun B, Du J, Wang J (2022) Alterations and mechanism of gut microbiota in graves' disease and hashimoto's thyroiditis. Pol J Microbiol 71(2):173–189