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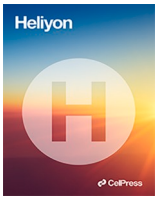

Research article

Synthetic bacterial consortia transplantation attenuates vaginal inflammation and modulates the immune response in a mouse model of Gardnerella vaginalis-induced bacterial vaginosis

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ABSTRACT

This study aimed to evaluate the efficacy of synthetic bacterial consortia transplantation (SBCT) and compare it with VMT (vaginal microbiota transplantation) in a mouse model of *Gardnerella vaginalis*-induced Bacterial vaginosis (BV). A murine model of *G. vaginalis*-induced BV was established, and mice were treated with SBCT, VMT, or saline. Histopathological changes, inflammatory cytokine levels, pro-inflammatory biomarker expression, helper T cell transcription factor expression, and vaginal microbiota composition were assessed. SBCT and VMT effectively suppressed *G. vaginalis* growth, reduced inflammation, and restored vaginal microbiota diversity. Both treatments attenuated epithelial damage, downregulated pro-inflammatory cytokines (IL-1β and IL-8), and upregulated the anti-inflammatory cytokine IL-10. SBCT and VMT also inhibited NF-κB activation, suppressed IL-17 expression, and enhanced Foxp3 expression in vaginal tissues. SBCT is a promising therapeutic approach for treating BV, as it effectively modulates the immune response and restores vaginal microbiota diversity in a mouse model of *G. vaginalis*-induced BV.

1. Introduction

Bacterial vaginosis (BV) is a common vaginal disorder characterized by a shift in the vaginal microbiota from a *Lactobacillus*-dominant community to a diverse array of anaerobic bacteria, such as *Gardnerella vaginalis*, *Atopobium vaginae*, and *Prevotella species* [1]. This dysbiotic state is associated with an increased risk of sexually transmitted infections, preterm birth, and pelvic inflammatory disease [2,3]. Current treatment options for BV, such as antibiotics, have limited long-term efficacy and can lead to recurrent infections [4]. Therefore, there is a pressing need for alternative therapeutic strategies that can effectively restore the healthy vaginal microbiota and prevent recurrent BV.

Lactobacillus species are the predominant bacteria in the vaginal microbiota of healthy women and play a crucial role in maintaining vaginal homeostasis [5,6]. These bacteria produce lactic acid, hydrogen peroxide, and bacteriocins, which create an inhospitable environment for pathogenic bacteria [7]. Several studies have investigated the potential of probiotics containing *Lactobacillus* strains for the treatment and prevention of BV [8]. However, the efficacy of these probiotics varies, and their ability to restore the

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complex vaginal microbiota remains limited [9].

Recent advances in microbiome research have led to the development of novel therapeutic approaches, such as fecal microbiota transplantation (FMT) and vaginal microbiota transplantation (VMT) [10]. These therapies aim to restore the healthy microbiota by transferring the entire microbial community from a healthy donor to the recipient [11]. VMT has shown promising results in treating recurrent BV and restoring the vaginal microbiota [12]. However, concerns regarding the safety and standardization of VMT remain, as the composition of the transplanted microbiota can vary between donors [13,14].

In response to these challenges, synthetic bacterial consortia transplantation (SBCT) has emerged as a promising alternative. SBCT involves the use of defined mixtures of known bacterial strains, designed to mimic the composition and function of the healthy microbiota while minimizing the risk of transferring pathogenic or antibiotic-resistant bacteria [15]. An important aspect of SBCT is the complex adaptation mechanism associated with microbes, which enables these consortia to dynamically adjust to the host environment and enhance the resilience and stability of the restored microbiota.These consortia can be designed to mimic the composition and function of the healthy microbiota while minimizing the risk of transferring pathogenic or antibiotic-resistant bacteria [16]. In the context of BV, a synthetic bacterial consortium comprising various *Lactobacillus* strains could provide a more targeted and reproducible approach to restore the vaginal microbiota [17,18].

In this study, we aimed to evaluate the efficacy of SBCT and compare it with VMT in a mouse model of *G. vaginalis*-induced BV. We hypothesized that SBCT, composed of four *Lactobacillus* strains isolated from healthy women, would effectively suppress *G. vaginalis* growth, reduce inflammation, and restore the vaginal microbiota diversity. Furthermore, we investigated the impact of SBCT and VMT on the local immune response and the expression of key pro-inflammatory biomarkers in the vaginal tissues. Our findings provide new insights into the potential of SBCT as a novel therapeutic approach for BV and contribute to the growing body of evidence supporting the use of microbiota-based therapies for vaginal disorders.

2. Materials and methods

2.1. Sample preparation and Lactobacillus cultivation

Lactobacillus strains were isolated from the vaginal discharge of five healthy women who provided informed consent. The institutional ethics committee of Yunnan Cancer Hospital approved the research (Ethics Number: Kmmu20230755). *L. gasseri*, *L. jensenii*, *L. acidophilus*, and *L. fermentum* isolates were selected for the construction of synthetic bacterial consortia. These strains were cultured aerobically in de Man, Rogosa, and Sharpe (MRS) broth at 37 °C for up to 24 h. The harvested bacteria were suspended in phosphate buffer solution (PBS; pH 7.0) at a density of 3×10^9 CFU mL^{-1}. For vaginal treatments, vaginal microbiota transplanted discharge from healthy female mice was diluted with PBS to a cell density of 3×10^8 CFU mL^{-1}.

2.2. Experimental cultivation of Gardnerella vaginalis (GV)

GV was cultured in Columbia Blood Agar Base Medium under anaerobic conditions using sealed anaerobic jars at 37 °C for up to 36 h [12]. The fermentation broth was harvested and suspended in PBS at a density of 3×10^9 CFU mL^{-1} for vaginal injection.

2.3. Experimental animal model and treatment Procedures

The animal experiments were conducted in compliance with national and international laws and policies, and were approved by the Laboratory Animal Care and Use Committee of Yunnan Cancer Hospital (Ethics Number: KMMU20230755). The research has been conducted according to the principles stated in the ARRIVE guidelines. At all stages, the experimenter was blind to the experimental groupings.

Five-week-old female BALB/c mice (Charles River, Beijing, China) were housed in individually ventilated cages under controlled temperature (22 ± 2 °C), humidity (55 ± 5 %), and a 12-h light/dark cycle. Inclusion/exclusion criteria were all pre-established and no samples or animals were excluded from the analysis. Mice were provided with standard chow diet and water ad libitum. Environmental enrichment included nesting material and plastic tunnels. Health monitoring was performed daily.

The mice were divided into four groups: Control (CON), GV-induced BV (GVI), GV-induced BV treated with synthetic bacterial consortia transplantation (SBCT), and GV-induced BV treated with vaginal microbiota transplantation (VMT). Six animals were allocated to each group (total animals: n = 24), the Ethical Committee decided the sample size. To induce pseudoestrus, mice in GVI, SBCT, and VMT groups were intraperitoneally injected with 0.3 mg of β-estradiol-3-benzoate in 100 μL of olive oil 3 days prior to GV treatment. Anesthetized mice (using 2–3% isoflurane inhalation) were inoculated with 3×10^9 CFU mL^{-1} *G. vaginalis* in 20 μL of cell suspensions via a mechanical suction tube into the vaginal cavity near the cervix, once every 24 h. The CON group received saline instead of the GV suspension. Bacterial burden was monitored by flat colony counting every 2 days post-induction.

GV-induced BV mice were treated daily with either synthetic bacterial consortia (20 μL, 3×10^9 CFU mL^{-1} in PBS) or vaginal microbiota transplantation (3×10^8 CFU mL^{-1}) for 14 days, while the GVI group received saline. Humane endpoints were established prior to the experiment, including rapid weight loss exceeding 20 % of initial body weight, inability to access food or water, signs of severe infection or pain, and prolonged lethargy or unconsciousness. No animals met these criteria during the study.

After treatment, mice were euthanized by cervical dislocation under deep anesthesia (5 % isoflurane inhalation). Vaginas were collected for mRNA extraction or histological inspection. Vaginal discharges were used for bacterial DNA extraction and high-throughput sequencing analysis.

2.4. Histopathological analysis of vaginal tissues

Vaginal tissues were fixed in 10 % neutral buffered formalin for 72 h, paraffin-embedded, and stained with hematoxylin and eosin (H&E). Histopathological changes were evaluated and compared among the CON, GVI, SBCT, and VMT groups.

2.5. Serum cytokine Quantification by ELISA

Serum was prepared from blood samples collected at the end of the treatment period. The concentrations of interleukin-1β (IL-1β), interleukin-8 (IL-8), and interleukin-10 (IL-10) were measured using commercial kits (Sino Best Biological Technology Co., Ltd., Shanghai, China) according to the manufacturer's protocol. Samples were diluted in assay buffer to adjust the concentration to the linear range of the standard curve, and serum from the CON group served as a control.

2.6. Quantitative analysis of gene expression

Total RNA was isolated from vaginal tissue using TRIzol® reagent (Vazyme, Nanjing, China), and cDNA was synthesized using a PrimeScriptTM RT Reagent Kit with gDNA Eraser (Vazyme, Nanjing, China). Real-time PCR analysis was performed for TNF-α, IL-17, Foxp3, and GAPDH (Table 1), with samples from the CON group serving as controls. RT-qPCR reactions were performed using Power SYBR green PCR master mix, and relative gene expression changes were calculated using the ΔΔCt method.

2.7. Analysis of vaginal microbiota composition and diversity

The V3-V4 region of the 16S rRNA gene was amplified from the extracted genomic DNA using the universal primers 338F and 806R. PCR amplification was performed in a two-step process. The first round of PCR was carried out in a 50-μL reaction mixture containing high-fidelity DNA polymerase, buffer, primers, enhancer, dNTPs, and template DNA. The second round of PCR was performed using the product from the first round as a template, with a reduced reaction volume and cycle number. The final PCR products were quantified, pooled, and subjected to high-throughput sequencing on the Illumina HiSeq 2500 platform. Bioinformatic analyses were conducted using the Majorbio Cloud Platform.

2.8. Statistical analysis

Each experiment was performed in triplicate and data were evaluated by mean ± standard error of the mean. Statistical significance was analyzed using one-way ANOVA, GraphPad Prism software, and unpaired t-tests. For non-normally distributed data, the nonparametric Kruskal-Wallis test was used, followed by Dunn's multiple comparison tests for pairwise comparisons ($P < 0.05$).

3. Results

3.1. Establishment of the G. vaginalis-induced bacterial vaginosis mouse model

The murine model of *G. vaginalis*-induced bacterial vaginosis was successfully established by intravaginal inoculation of *G. vaginalis* (2×10^9 CFU mL^{-1}) for 8 days. Mice in the *G. vaginalis*-inoculated (GVI) group exhibited swollen, reddened vaginas and increased vaginal discharge compared to the CON group, despite no significant changes in behavior, fecal consistency, or body weight. Quantitative analysis revealed a continuous increase in *G. vaginalis* burden in the vaginal discharge of GVI mice from day 4 to day 8 post-inoculation, reaching a stable level by day 10, while no *G. vaginalis* was detected in the CON group (Fig. 1). Following the induction of vaginitis, GVI mice were divided into three treatment groups: SBCT, VMT, and saline control, administered daily for 2 weeks.

3.2. Histopathological evaluation of vaginal tissues in bacterial vaginosis mice

H&E stained vaginal tissues was performed to assess the therapeutic effects of SBCT and VMT on *G. vaginalis*-induced vaginitis in mice. Fig. 2a showed the CON group with normal vaginal epithelial tissue, minimal inflammatory cell presence, and typical epithelial stratification. Compared to the CON group, GVI mice exhibited thickened vaginal epithelial tissue, increased inflammatory cell infiltration, and significant epithelial stratification (Fig. 2b). After 2 weeks of treatment with SBCT, vaginal epithelial mucosa showed marked improvement, with reduced stratification and cellular infiltration (Fig. 2c). Notably, VMT-treated mice demonstrated greater

Table 1
Primer sequences for real-time PCR analysis.

Target Gene	Forward Primer Sequence (5'→3')	Reverse Primer Sequence (5'→3')	Product Size (bp)
TNF-α	5′-CAGCCTCTTCTCCTTCCTGAT-3′	5′-GCTGCTGTTTCCACATCTCC-3′	210
IL-17	5′-CCTCAAAGCTCAGCGTGTCC-3′	5′-GCTGAGCTTTGAGGGATGATG-3′	156
Foxp3	5′-CCCAGGAAAGACAGCAACCTT-3′	5′-TTCTCACAACCAGGCCACTTG-3′	122
GAPDH	5′-TGCACCACCAACTGCTTAGC-3′	5′-GGCATGGACTGTGGTCATGAG-3′	143

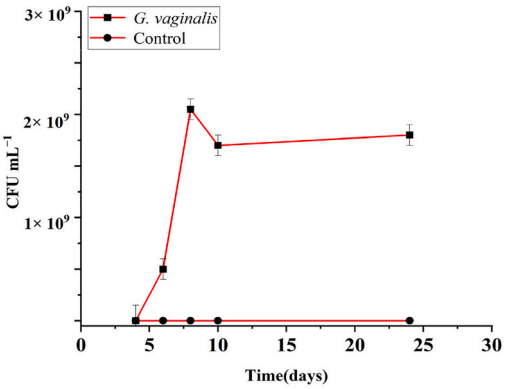

Fig. 1. Longitudinal assessment of *G. vaginalis* burden in vaginal lavage fluids. Bacterial load was quantified on days 4, 6, 8, 10 and 21 post-inoculation. Data are presented as mean ± standard error of the mean from three independent experiments. Number of mice in each group = 6 (total animals: n = 12).

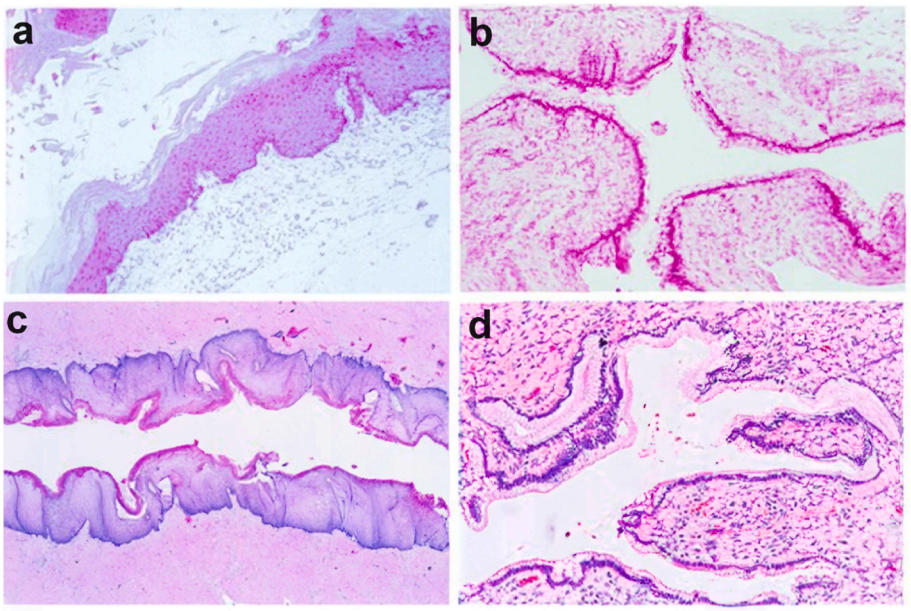

Fig. 2. Histopathological analysis of H&E stained vaginal tissues in a mouse model of *G. vaginalis*-induced vaginitis. (**a**) CON, normal control mice; (**b**) GVI, *G. vaginalis-infected* mice; (**c**) SBCT, mice treated with synthetic bacterial consortia; (**d**) VMT, mice treated with vaginal microbiota transplantation. Images were captured using a Pannoramic MIDI digital section scanner at 100 × magnification; Scale bar: 1 μm; Number of mice in each group = 6 (total animals: n = 24).

epithelial damage recovery than SBCT-treated mice when compared to the CON group (Fig. 2d).

3.3. Inflammatory cytokine profile in serum samples

Serum levels of pro-inflammatory cytokines, IL-1β and IL-8, and the anti inflammatory cytokine, IL-10, were measured by ELISA to evaluate the immune response in *G. vaginalis*-induced bacterial vaginosis. GVI mice exhibited significantly elevated IL-1β and IL-8 levels compared to the CON group (Fig. 3a). Similarly, Fig. 3b illustrates that IL-8 levels were significantly higher in GVI mice than in the CON group, further confirming the inflammatory state induced by *G. vaginalis* (Fig. 3b). Conversely, as depicted in Fig. 3c, the anti-inflammatory cytokine IL-10 was found to be decreased in GVI mice when compared to the CON group. This trend was reversed following treatment with SBCT or VMT, where an enhancement in IL-10 expression was observed, indicating a shift towards an anti-inflammatory state. (Fig. 3c).

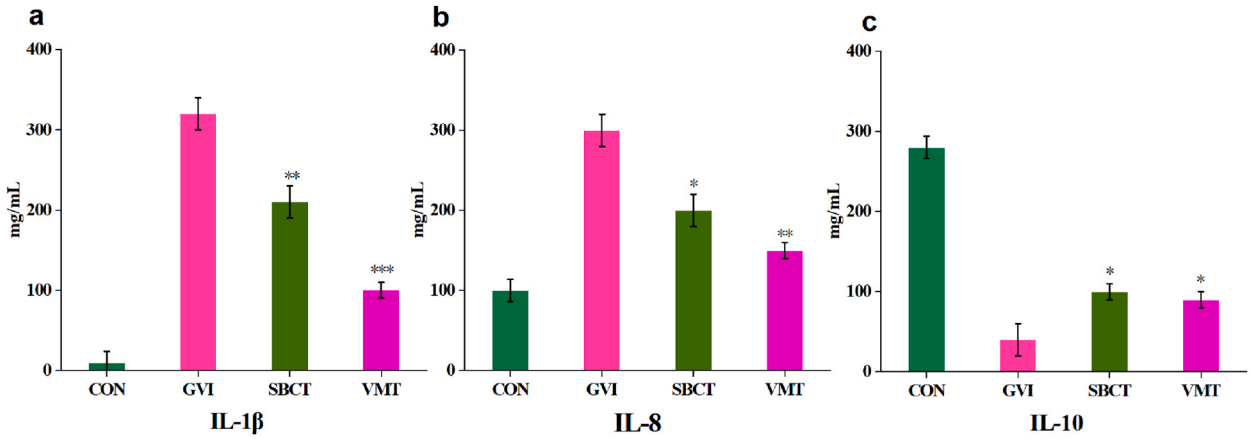

Fig. 3. Serum levels of inflammatory cytokines in different experimental groups. (**a**) IL-1β, interleukin-1β; (**b**) IL-8, interleukin-8; (**c**) IL-10, interleukin-10. CON, normal control mice; GVI, *G. vaginalis-infected* mice; SBCT, mice treated with synthetic bacterial consortia; VMT, mice treated with vaginal microbiota transplantation. Data are presented as mean ± standard error of the mean from three independent experiments. Number of mice in each group = 6 (total animals: n = 24). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. GVI group.

3.4. Expression of pro-inflammatory biomarkers in vaginal tissues

The involvement of the innate immune system in the anti-bacterial vaginosis mechanism of SBCT and VMT was investigated by assessing the activation of NF-κB and the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in vaginal tissues. RT-PCR analysis revealed significantly increased expression of TNF-α (Fig. 4a), a downstream marker of NF-κB activation, as well as iNOS (Fig. 4b) and COX-2 in the vaginal tissues of GVI mice (Fig. 4c). However, SBCT and VMT treatment effectively downregulated the expression of these pro-inflammatory genes (Fig. 4), indicating their ability to inhibit NF-κB activation and modulate the innate immune response in G. vaginalis-induced bacterial vaginosis.

3.5. Transcription factor expression of helper T cells in vaginal tissues

The impact of SBCT and VMT on the adaptive immune response was evaluated by examining the expression of helper T cell transcription factors in vaginal tissues. GVI mice exhibited significantly upregulated expression of interleukin-17 (IL-17). However, following treatment with SBCT or VMT, there was a marked suppression of IL-17 expression, suggesting that these treatments can modulate the adaptive immune response by reducing pro-inflammatory cytokine production (Fig. 5a). Contrary to IL-17, Forkhead Box Protein P3 (Foxp3) expression was downregulated in GVI mice, which is consistent with a diminished regulatory T cell response. Post-treatment analysis showed an enhancement of Foxp3 expression in both SBCT and VMT groups, indicating a potential restoration of regulatory T cell function (Fig. 5b).

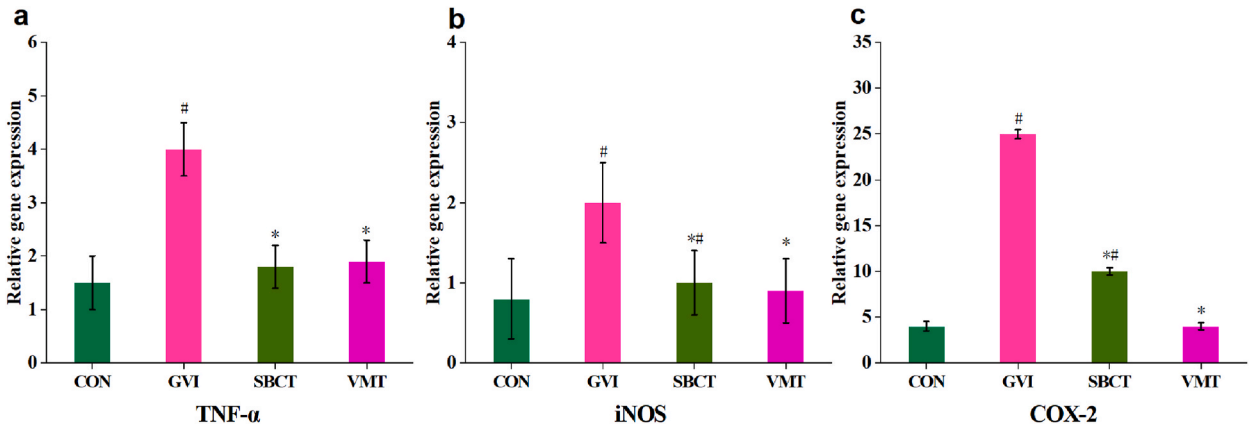

Fig. 4. Relative gene expression of pro-inflammatory biomarkers in vaginal tissues. (**a**) TNF-α, tumor necrosis factor-α; (**b**) iNOS, inducible nitric oxide synthase; (**c**) COX-2, cyclooxygenase 2. CON, normal control mice; GVI, *G. vaginalis-infected* mice; SBCT, mice treated with synthetic bacterial consortia; VMT, mice treated with vaginal microbiota transplantation. Data are presented as mean ± standard error of the mean from three independent experiments. Number of mice in each group = 6 (total animals: n = 24). $^{\#}P < 0.05$ vs. normal control group; *$P < 0.05$ vs. GVI group.

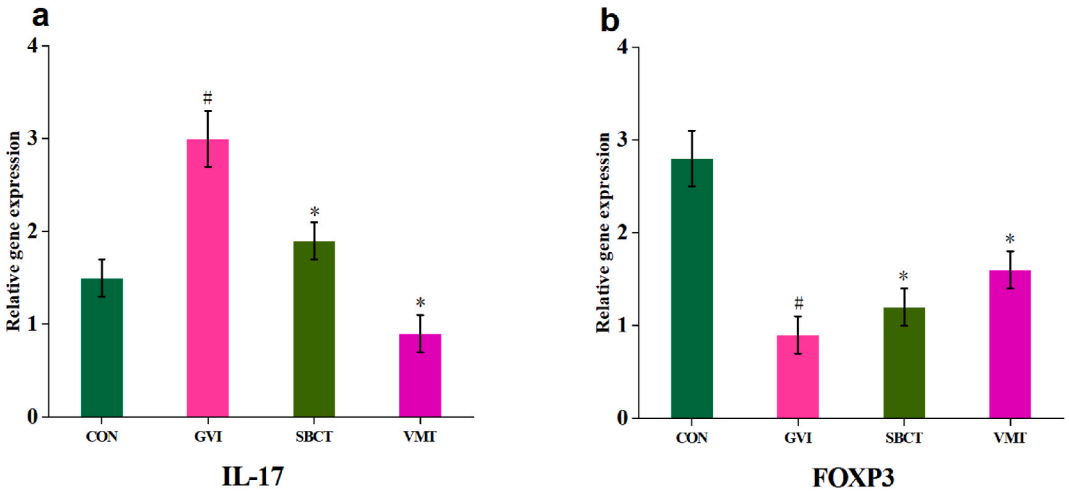

Fig. 5. Relative gene expression of helper T cell transcription factors in vaginal tissues. (**a**) IL-17, interleukin-17; (**b**) FOXP3, Forkhead Box Protein P3. CON, normal control mice; GVI, G. vaginalis-infected mice; SBCT, mice treated with synthetic bacterial consortia; VMT, mice treated with vaginal microbiota transplantation. Data are presented as mean ± standard error of the mean from three independent experiments. Number of mice in each group = 6 (total animals: n = 24). $^{\#}P < 0.05$ vs. normal control group; $^{*}P < 0.05$ vs. GVI group.

3.6. Vaginal microbiota composition and diversity

High-throughput sequencing analysis was performed to assess the impact of SBCT and VMT on the vaginal microbiota composition and diversity in *G. vaginalis*-induced bacterial vaginosis. The CON group has 79 unique operational taxonomic units (OTUs), indicating a diverse and healthy vaginal microbiota in the absence of *G. vaginalis* infection (Fig. 6a). The GVI group shows a significant reduction in unique OTUs with only 3, suggesting a loss of microbial diversity due to the *G. vaginalis*-induced BV. The SBCT group has 28 unique OTUs, which implies that the synthetic bacterial consortia have introduced a variety of beneficial bacteria, enhancing the microbial diversity. The VMT group displays 14 unique OTUs, indicating that the transplanted vaginal microbiota also contributes to increasing the microbial diversity, although to a lesser extent than SBCT. There are 19 OTUs shared by all groups, representing the core microbiota that is common across different conditions. The presence of unique OTUs in the treatment groups (SBCT and VMT) compared to the GVI group suggests that both treatments are effective in modulating the vaginal microbiota and restoring diversity disrupted by *G. vaginalis* infection. The higher number of unique OTUs in the SBCT group may indicate a more robust restoration of the microbiota, potentially offering a more effective treatment for BV.

The Chao1 index revealed higher microbial diversity in the treatment groups (SBCT and VMT) and the CON group compared to the GVI group (Fig. 6b). The Shannon index also indicated significant differences in microbial diversity between the treatment groups and the GVI group (Fig. 6c). These findings suggest that SBCT and VMT can effectively modulate the vaginal microbiota composition and restore microbial diversity in G. vaginalis-induced bacterial vaginosis.

4. Discussion

In this study, we demonstrated the efficacy of SBCT in treating G. vaginalis-induced BV in a mouse model. Our results showed that SBCT effectively suppressed *G. vaginalis* growth, reduced inflammation, and restored the diversity of the vaginal microbiota. These findings highlight the potential of SBCT as a novel therapeutic approach for BV and provide new insights into the mechanisms underlying the restoration of vaginal homeostasis.

The murine model of *G. vaginalis*-induced BV used in this study has been well-established and widely used to investigate the pathogenesis and treatment of BV [19–21]. Our results confirmed the successful establishment of the model, as evidenced by the increased *G. vaginalis* burden, vaginal inflammation, and altered cytokine profile in the GVI group. The continuous increase in *G. vaginalis* load from day 4 to day 8 post-inoculation and the subsequent stabilization by day 10 are consistent with previous reports [22,23], indicating that this model accurately mimics the clinical course of BV.

Histopathological evaluation of vaginal tissues revealed that SBCT and VMT effectively attenuated the G. vaginalis-induced epithelial damage and inflammation. The reduced epithelial stratification and cellular infiltration observed in the treated groups suggest that both therapies promote tissue repair and resolution of inflammation [24]. Interestingly, VMT demonstrated greater epithelial recovery compared to SBCT, which may be attributed to the more complex and diverse microbial community present in the VMT inoculum [12]. However, the significant improvement observed in the SBCT group highlights the potential of rationally designed bacterial consortia in restoring vaginal health.

The analysis of inflammatory cytokines in serum samples provided further evidence for the anti-inflammatory effects of SBCT and VMT. The elevated levels of IL-1β and IL-8 in the GVI group are consistent with the pro-inflammatory response associated with BV [25].

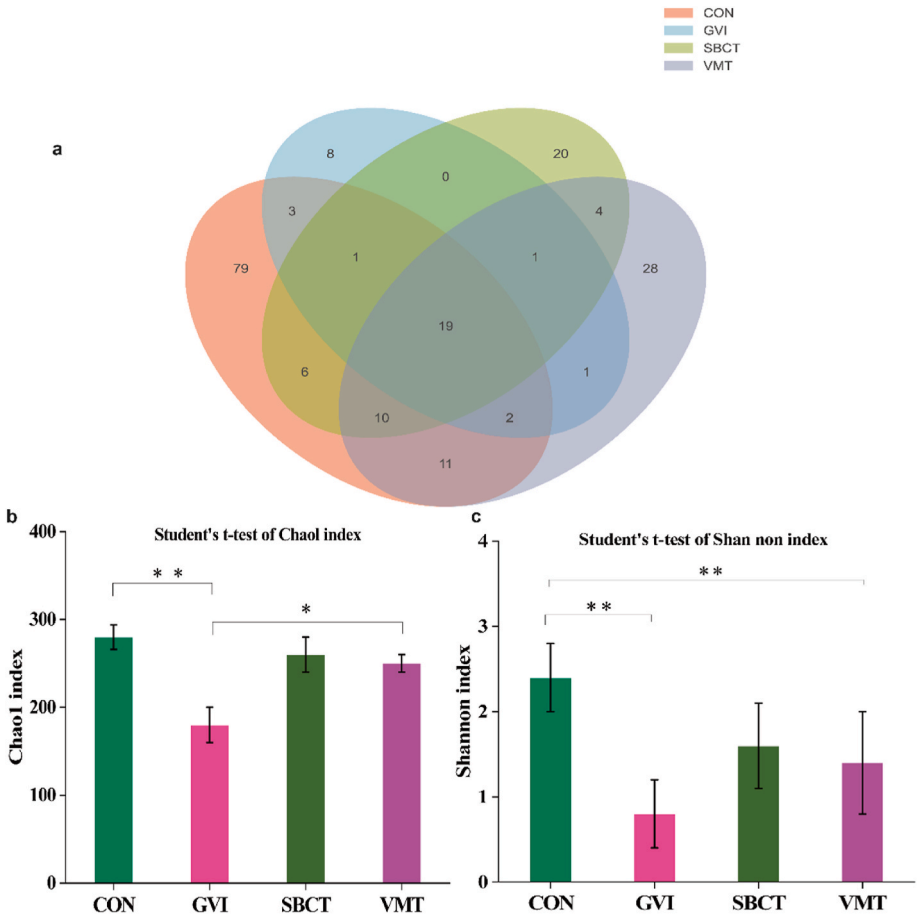

Fig. 6. Assessment of vaginal microbiota diversity in mice with vaginal dysbiosis using high-throughput sequencing. (**a**) Scalar Venn representation of shared and unique OTUs among the experimental groups; (**b**) Chao1 index; (**c**) Shannon index. CON, normal control mice; GVI, G. vaginalis-infected mice; SBCT, mice treated with synthetic bacterial consortia; VMT, mice treated with vaginal microbiota transplantation. Data are presented as mean ± standard error of the mean from three independent experiments. Statistical analyses were performed using one-way ANOVA for normally distributed data and the Kruskal-Wallis test followed by Dunn's multiple comparison tests for non-normally distributed data. Number of mice in each group = 6 (total animals: n = 24). Statistical significance was determined using one-way ANOVA for normally distributed data and the Kruskal-Wallis test with Dunn's multiple comparison tests for non-normally distributed data, with results presented as mean ± standard error of the mean from three independent experiments, where *P < 0.05 and **P < 0.01 denote significant differences.

The suppression of these cytokines and the concomitant increase in the anti-inflammatory cytokine IL-10 in the treated groups indicate that both therapies modulate the systemic immune response towards a more tolerogenic state [26,27]. These findings suggest that the restoration of vaginal microbiota diversity by SBCT and VMT plays a crucial role in regulating the host immune response and maintaining vaginal homeostasis [28].

The involvement of the innate immune system in the anti-BV effects of SBCT and VMT was further investigated by assessing the expression of pro-inflammatory biomarkers in vaginal tissues. The increased expression of TNF-α, iNOS, and COX-2 in the GVI group is consistent with the activation of NF-κB signaling and the subsequent production of pro-inflammatory mediators [29]. The downregulation of these biomarkers in the treated groups suggests that SBCT and VMT inhibit NF-κB activation and attenuate the local inflammatory response [30]. These findings highlight the importance of the vaginal microbiota in modulating the innate immune response and maintaining vaginal health.

In addition to the innate immune response, we also investigated the impact of SBCT and VMT on the adaptive immune response by examining the expression of helper T cell transcription factors in vaginal tissues. The upregulation of IL-17 and downregulation of Foxp3 in the GVI group indicate a shift towards a pro-inflammatory Th17 response and a suppression of regulatory T cell (Treg) activity [31]. The reversal of this pattern in the treated groups suggests that SBCT and VMT promote a more balanced Th17/Treg response, which is crucial for maintaining mucosal homeostasis and preventing excessive inflammation [32]. These findings provide new insights into the immunomodulatory effects of microbiota-based therapies and their potential to restore the balance between pro- and anti-inflammatory responses in the vaginal mucosa [33].

The high-throughput sequencing analysis of the vaginal microbiota revealed that SBCT and VMT effectively restored the microbial diversity and composition in *G. vaginalis*-induced BV. The increased diversity indices in the treated groups compared to the GVI group

are consistent with previous reports demonstrating the ability of microbiota-based therapies to restore the complex vaginal microbial community [34]. The enrichment of *Lactobacillus* species and the reduction of pathogenic bacteria, such as *Escherichia-Shigella* and *Vagococcus*, in the treated groups further support the role of SBCT and VMT in reestablishing a healthy vaginal microbiota [28]. These findings highlight the potential of rationally designed bacterial consortia in restoring the ecological balance of the vaginal microbiome and preventing the overgrowth of pathogenic bacteria [5].

While both SBCT and VMT demonstrated significant efficacy in treating *G. vaginalis*-induced BV, it is important to acknowledge the limitations of this study. First, the mouse model, although widely used, may not fully recapitulate the complex microbial and immunological interactions in the human vaginal environment [19]. Second, the use of a single pathogen, *G. vaginalis*, to induce BV may not capture the full spectrum of microbial dysbiosis associated with the disorder [35]. Future studies should investigate the efficacy of SBCT in more complex models of BV, such as those involving multiple pathogenic bacteria or clinical isolates from BV patients [28].

In conclusion, our study demonstrates the efficacy of synthetic bacterial consortia transplantation in treating *G. vaginalis*-induced bacterial vaginosis in a mouse model. SBCT effectively suppressed *G. vaginalis* growth, reduced inflammation, and restored the diversity and composition of the vaginal microbiota. These effects were mediated through the modulation of both innate and adaptive immune responses, as evidenced by the downregulation of pro-inflammatory biomarkers and the restoration of a balanced Th17/Treg response. While VMT showed greater efficacy in certain aspects, the significant improvement observed in the SBCT group highlights the potential of rationally designed bacterial consortia as a novel therapeutic approach for BV. Future studies should focus on optimizing the composition of SBCT, evaluating its long-term efficacy and safety, and investigating its potential for preventing recurrent BV. Furthermore, the immunomodulatory effects of SBCT warrant further investigation, as they may have broader implications for the treatment of other mucosal disorders characterized by microbial dysbiosis and chronic inflammation. Artificial intelligence (AI) and machine learning (ML), including deep learning, offer powerful tools for analyzing complex biological data in microbiota-based therapies. They enhance our understanding of microbial communities and host interactions, aid in identifying novel microbial biomarkers, predict disease states, and guide the design of synthetic bacterial consortia with optimized therapeutic potential [36].

Data availability statement

The high-throughput sequencing data generated in this study are not currently publicly available due to ongoing analysis for future studies. However, derived data supporting the findings of this study are expected to be made available from the corresponding author upon reasonable request and with permission after June 2025, following the completion and publication of these ongoing studies. This timeline is subject to the successful conclusion of our ongoing research projects. Interested researchers may contact the corresponding author for updates on data availability status and any potential changes to this timeline.

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CRediT authorship contribution statement

Ying Liu: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Liang He:** Software, Methodology. **Yan Hu:** Software, Methodology. **Xingya Liao:** Software. **Hongyan Wang:** Writing – review & editing, Funding acquisition. **Linlin Yang:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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