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ORIGINAL RESEARCH

The Succession of Cervical Canal Microbiota in Endometrial Cancer and Cervical Cancer: A Clinical Metagenomics Study

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Objective: To define stage-specific cervical canal microbiota signatures across the continuum of gynecologic malignancies from benign endometrial cancer (BE)/cervical cancer precancerous lesions (CIN) to endometrial cancer (EC)/cervical cancers (CC), and to evaluate their potential as diagnostic biomarkers and therapeutic targets.

Methods: In the observational study, metagenomic sequencing was employed to investigate the cervical canal microbiota of 45 patients, including BE, EC, CIN, and CC. Specimen collection was performed by an experienced physician. All samples were sequenced utilizing the shotgun approach. The microbial statistical analyses were conducted using R.

Results: Compared to the non-cancerous group (BE and CIN), the index related to microbial community stability decreases significantly and the incidence of cervical canal dysbiosis increases in the cancerous group (EC and CC). Microbial diversity exhibited significant differences between BE and EC, CIN and CC, as well as cancerous and non-cancerous groups. At the species level, some species were significantly decreased (eg, *Lactobacillus iners*) and increased (eg, *Staphylococcus haemolyticus, Pasteurella multocida, Pseudomonas putida*, and other opportunistic pathogen) in the cancerous group.

Conclusion: The cervical canal represents a distinct microbial niche, with its dysbiotic progression reflecting the trajectory of oncogenic transformation. The progression from non-cancerous to cancerous states is characterized by the replacement of the vaginal microbial community, which is dominated by *Lactobacillus iners*, with a gradual shift towards opportunistic pathogen. Disease diagnosis and complementary therapies focused on lactobacilli and hallmark opportunistic pathogen may offer new insights for precision oncology.

Keywords: community succession, cervical canal microbiota, microbial dysbiosis, endometrial cancer, cervical cancer

Introduction

Endometrial cancer (EC) and cervical cancer (CC) rank among the most prevalent gynecological malignancies globally, imposing significant clinical and societal burdens.[1] In China alone, according to the estimates on the prevalence of malignant tumors in 2022, EC accounts for 150,700 new cases and CC is expected to have 77,700 new cases, both ranking within the top 10 malignancies affecting women.[2] While EC arises from the endometrial epithelium and frequently presents with postmenopausal bleeding (>90% of cases),[3,4] CC originates from cervical epithelial cells and is strongly associated with persistent human papillomavirus (HPV) infection.[5] Despite advances in therapeutic strategies, both cancers continue to exhibit high morbidity and mortality rates, underscoring the necessity for deeper insights into their underlying mechanisms and development of personalized interventions.

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The etiological divergence between EC and CC is striking. EC risk factors include obesity, reproductive and metabolic disorders, and genetic predisposition,[6] whereas CC is predominantly linked to HPV persistence and immune evasion mechanisms.[7] While these distinct etiological pathways have traditionally guided clinical management, the emergence of precision oncology has redefined screening and therapeutic paradigms by bridging molecular mechanisms with targeted interventions (eg, molecular subtyping,[8] immune checkpoint inhibitors,[9] and non-coding RNA[10]). However, critical challenges persist: chemotherapy resistance, limited druggability of key genetic drivers, absence of evidence-based screening tools for the vast majority of cancer types.[11] These limitations highlight the need for novel biomarkers and complementary therapies to refine precision strategies. Among emerging diagnosis and complementary therapies, the reproductive tract microbiota emerges as a promising candidate due to its dual role in immune modulation and tissue homeostasis.

Emerging evidence suggests that the reproductive tract microbiota—a critical mediator of local immunity and tissue homeostasis— plays an significant role.[12,13] The female reproductive tract microbiota, particularly in the lower tract, is dominated by *Lactobacillus spp*. (*L. crispatus, L. gasseri, L. jensenii*) under healthy conditions.[14,15] These commensals maintain vaginal acidity through lactic acid production, inhibit pathogens via bacteriocins and hydrogen peroxide, and modulate immune responses.[16] Dysbiotic shifts characterized by reduced Lactobacilli abundance and increased microbial diversity have been associated with gynecological conditions such as bacterial vaginosis and pelvic inflammatory disease.[17,18] Recent studies further suggest that such dysbiosis may contribute to carcinogenesis by inducing chronic inflammation, disrupting epithelial barriers, and altering metabolic microenvironments.[12,19] The cervical canal, anatomically connecting the uterus and vagina, represents a strategic microenvironment for biomarker discovery. Microbial profiling at this junction may yield novel targets for refining precision strategies in gynecologic oncology. While the oncogenic potential of microbiota has been explored in other cancer types, comprehensive studies employing metagenomic analysis to explore the cervical canal microbiota across various stages of EC and CC remain scarce at the species level. Specifically, understanding the metagenomic features of the cervical canal microbiota at various disease stages —benign endometrial lesions (BE), EC, cervical intraepithelial neoplasia (CIN), and CC —could illuminate microbial characteristics associated with disease progression and offer insights for innovative diagnostic and therapeutic strategies.

To address this, we conducted a metagenomic study of cervical canal microbiota in 45 patients spanning four clinical stages: BE, EC, CIN, and CC. By elucidating the relationship between changes in microbial community composition and cancer development, this study aims to elucidate the intricate interactions between microbiota and cancer and propose microbiota-targeted strategies. Our findings may advance the integration of microbial ecology into precision frameworks, ultimately improving therapeutic efficacy and outcomes for patients for gynecologic malignancies.

Materials and Methods

Sample Collection

To investigate the cervical canal microbiota in patients with endometrial and cervical cancer, cervical canal samples were collected from 45 patients at Hunan Cancer Hospital according to strict inclusion criteria. The cohort comprised 11 patients with BE, 6 with EC, 8 with CIN, and 20 with CC. All participants were treatment-naive at the time of sampling, with specimens collected during initial diagnostic workup prior to surgical intervention, chemotherapy, or radiation. Patients receiving hormonal therapy, antibiotics, or probiotics within 3 months were excluded. All participants provided informed consent and had complete clinical and pathological data. The present study was approved by the Ethics Committee of Hunan Cancer Hospital (KYJJ-2023-025) and followed the Declaration of Helsinki on Biology for Human Trials. Specimen collection was performed by an experienced physician of Hunan Cancer Hospital, from July 2023 to January 2024. After wiping secretions from the cervical surface, the swab was probed into the cervical canal and rotated 6–8 turns clockwise before being removed. All swabs were immediately broken into cryotubes with 500 μL DNA storage solution, and the cryotubes were stored at −80°C for further analysis.

DNA Extraction and Metagenome Sequencing

All samples were subjected to metagenomic sequencing utilizing the shotgun approach at Shanghai Biozeron Biotech Co., Ltd (China). The E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, USA) was employed to extract total DNA from each cervical canal

sample. The UV spectrophotometer, UItrospec 2100 pro (Amersham Biosciences, USA), was employed to quantify DNA yield, and DNA purity was evaluated via agarose gel electrophoresis of metagenomic DNA. Subsequently, qualified genomic DNA (standards: concentration ≥ 10 ng/μL, total amount above 2ug, DNA must have a clear main band, no degradation/RNA/protein/other impurities contamination) was randomly fragmented into 200–500 bp segments using ultrasound (Covaris S220, USA), with fragment size assessed by agarose gel electrophoresis, and the electrophoresis products were recovered using the QIAquick Gel Extraction kit. The construction of the PE library went through the following procedures: (1) blunting DNA ends, (2) purifying PCR products with QIAquick 8 PCR Purification Kit and MinElute PCR Purification Kit, (3) amplifying the purified DNA fragments to obtain a DNA fragment of 300 to 350 bp with QIAquick Gel Extraction, (4) smoothing and repairing the DNA ends. Finally, the library was purified and sequenced on the Illumina NovaSeq 6000 PE250 platform at Shanghai Biozeron Biotech Co., Ltd., and the obtained sequencing data underwent quality control. Amplify and purify the DNA fragment to obtain a DNA fragment of 300 to 350 base pairs.

Quality Control and Species Annotation

To obtain high-quality filtered data, we performed stringent quality control on the raw data to remove contamination and human host sequences. Initially, Fastp (version 0.23.4) was employed to filter out low-quality sequences and eliminate adapter contamination.[20] Subsequently, FastUniq (version 1.1.0) was utilized to identify and remove duplicate sequences within paired short reads.[21] Next, Bowtie2 (version 12.3.0) was employed to align the sequences against the human genome database (hg38) to filter out sequences of human origin, thereby obtaining microbial sequences.[22] Kraken2 (version 2.1.3) was then used to annotate the genetic sequences against a microbial standard database to obtain taxonomic units (unique identifiers of NCBI classification) for each gene.[23] Subsequently, these taxonomic identifiers and names were translated into corresponding species information ranging from phylum to species level. Bracken (version 2.9) was used to statistically analyze the abundance and relative abundance of species.[24]

Data Analysis and Visualization

The data analysis and visualization process were outlined in Supplementary 1. The downstream microbial statistical analyses were conducted using R (version 4.3.1). The normality of data was assessed using the Shapiro–Wilk test, and variance equality was checked using Levene's test. If both tests yielded $P > 0.05$, differences were analyzed using t-tests. Otherwise, differences were assessed using the Wilcox test. Unless specified otherwise, all figures were generated using the ggplot2 package. Initially, R and Gephi (version 0.10.1) software were employed to characterize microbial community structures and co-occurrence across four case groups. The specific steps are as follows: (1) all OTUs were screened to remove those with an average relative abundance lower than 0.01% and those occurring in fewer than one-fifth of the total samples. (2) Spearman correlation analysis was then performed on the OTUs, and those with a p-value > 0.001 or $|r| < 0.60$ were excluded. (3) A weighted undirected graph was constructed based on the correlation coefficient matrix, and isolated nodes with a degree of 0 were removed. (4) edge attributes (corr, weight) were assigned to the network, and taxonomic information was added to the nodes. (5) Visualization was performed using Gephi. Next, α-diversity was assessed using the Shannon, Chao1, and Pielou indices based on generated species composition tables. β-diversity was analyzed using Principal Coordinates Analysis (PCOA) based on Bray-Curtis distances. Furthermore, Linear Discriminant Analysis Effect Size (LEfSe) and limma analyses were utilized to identify potential biomarkers that most likely explain inter-group microbial differences. Pearson correlation analysis was employed to explore interaction relationships among different species. Linear regression was used to display dynamic changes among these microbial differences.

Results

The Cervical Canal Microbiota Characteristics Across Disease States

Microbial co-occurrence networks were employed to illustrate the characteristics and structural reorganization of microbial ecosystems. In which, each node represents a microbial species, and the edges indicates a specific relationship between microorganisms. This analysis revealed progressive topological destabilization associated with oncogenic progression (Figure 1A–D). When comparing non-cancerous groups (BE and CIN) to cancer groups (EC and CC), we observed that while the non-cancerous groups exhibited heightened connectivity (Figure 1A and C), this hyperconnected

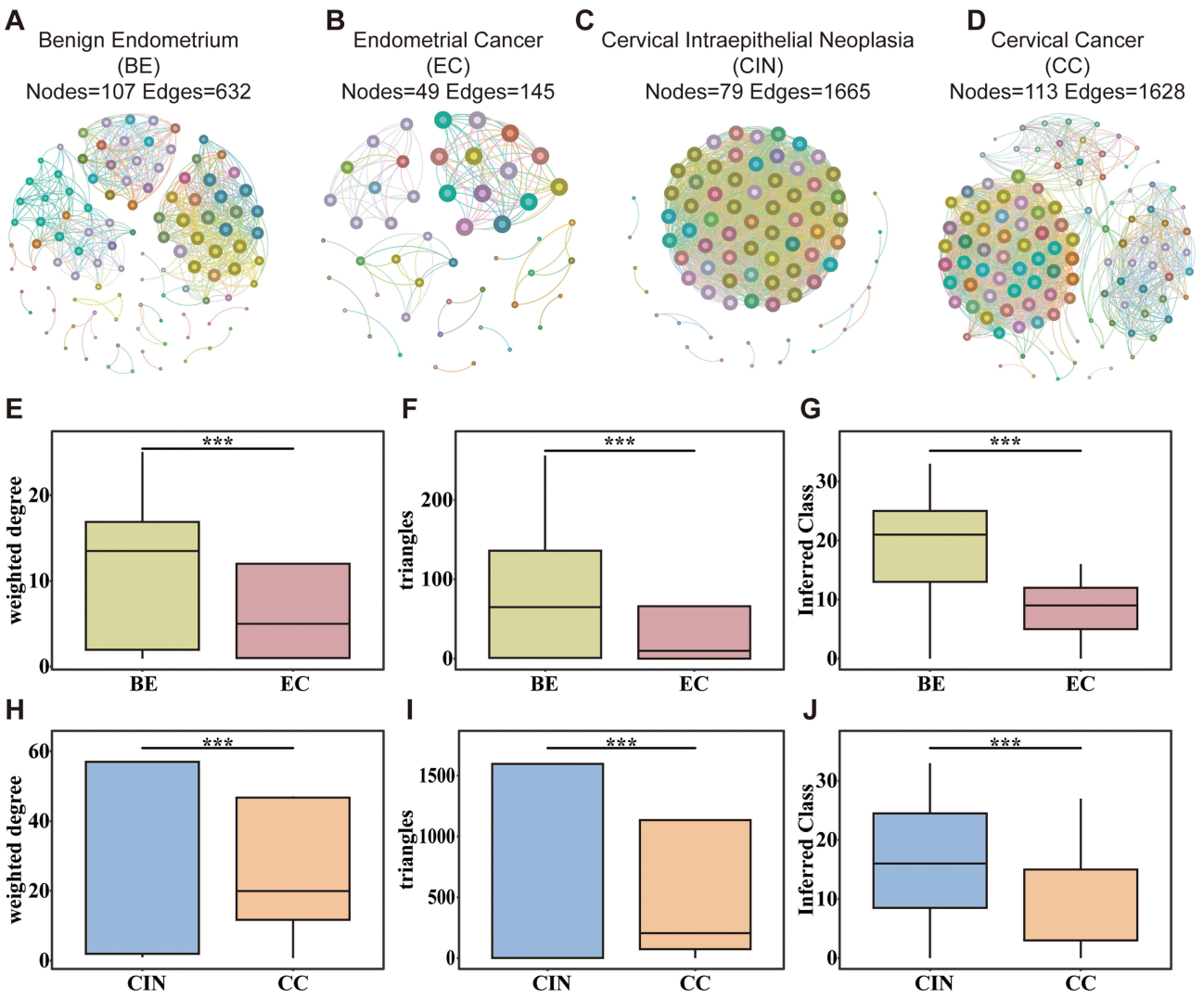

Figure 1 The Co-occurrence network analysis of microbiota in the four groups. (**A**) Network of BE. (**B**) Network of group EC. (**C**) Network of group CIN. (**D**) Network of group CC. Comparison of network topology properties among groups, under the same network construction parameters (the absolute value of R is less than 0.6, P < 0.01), the more weighted degree, triangles, and inferred class (**E–J**) appeared in the network diagram, the more complex the network was. (Differential analysis was performed by Wilcoxon test, ***P < 0.001).

state collapsed dramatically in cancer groups (Figure 1B and D). Each module represents a relatively independent sub-network or functional group, within which microorganisms exhibit strong interactions with one another. This architectural transformation was accompanied by progressive erosion of modular organization, with functional sub-network counts declining from 13 modules in BE and 11 in CIN to 12 in EC and merely 6 in CC. This progressive loss of modular organization parallels tumor progression stages. Accordingly, network characteristic parameters were calculated for each group. The results revealed significant reductions in network parameters, including weighted degree measuring connection strength, triangle reflecting clustering, and inferred class assessing organizational complexity ($P < 0.001$) (Figure 1E–J) in the cancer groups relative to the precancerous and benign groups. This implies that the non-cancerous groups (BE and CIN groups) demonstrate greater total connectivity and complexity relative to the cancerous groups (EC and CC groups). Network clustering in the non-cancerous groups was significantly greater than in the cancerous groups. Thus, compared to precancerous lesions and benign tumors, the cervical canal microbiota interactions in the cancer groups demonstrate significantly reduced connectivity and diminished stability.

Diversity Analysis of the Cervical Canal Microbiota

To investigate the microbial composition at various stages of endometrial and cervical cancers, a total of 1,878 species were identified across all samples. In the endometrial group, a total of 1,739 species were identified, including 1,508 unique species in the BE group and 27 unique species in the EC group. In the cervical group, a total of 855 species were identified, including 118 unique species in the CIN group and 436 unique species in the CC group. The rarefaction curves of species diversity for all samples tended to plateau, indicating adequate sequencing depth. When comparing the BE and EC groups, the α-diversity index Shannon showed no significant difference ($P = 0.5952$) (Figure 2A), but the α-diversity index Chao1 was significantly lower in the EC group than the BE group ($P = 0.0202$) (Figure 2B). Similarly, in the CIN and CC groups, the α-diversity index Shannon showed no significant difference ($P = 0.1653$) (Figure 2C), but the α-diversity index Pielou was significantly lower in the CC group compared to the CIN group ($P = 0.0285$) (Figure 2D). PCoA and non-parametric multivariate analysis (adonis) revealed significant β-diversity differences between the endometrial groups ($P = 0.01$, $R^2 = 0.177$) (Figure 2E) and cervical groups ($P = 0.004$, $R^2 = 0.134$) (Figure 2F). The inter-group differences were greater than the intra-group differences, indicating significant diversity disparities between the BE and EC groups, as well as between the CIN and CC groups.

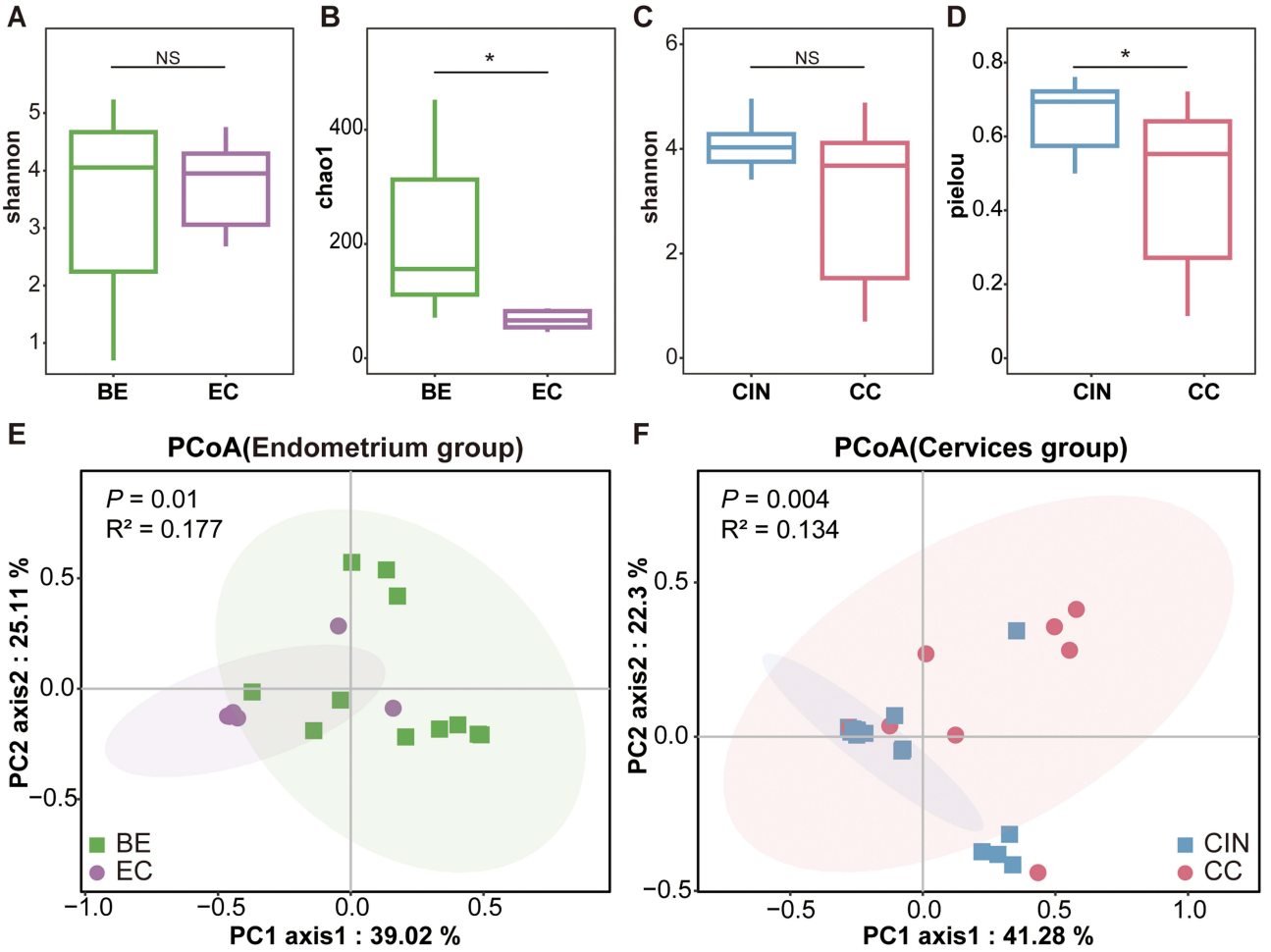

Figure 2 The diversity analysis of microbial community. Analysis of alpha diversity was presented by chao1 (**A** and **C**) and pielou (**B** and **D**). (Wilcoxon test, *P < 0.05). Beta diversity was presented by PCoA analysis based on the Bray–Curtis dissimilarity of the endometrium group (**E**) and cervices group (**F**). The confidence ellipse contained 95% of the samples in each group.

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Analysis of Microbial Community Composition in the Cervical Canal

To explore the variations in bacterial community composition in endometrial and cervical cancers, we compared the differences in relative abundance at the phylum and species levels among four groups.

Both the BE and EC groups, as well as the CIN and CC groups, exhibited similarities and differences in bacterial composition. At the phylum level, a total of 28, 11, 17, and 23 phyla were identified in the BE, EC, CIN, and CC groups, respectively. The non-cancerous group was predominantly composed of Firmicutes, whereas Proteobacteria predominated in the cancer group (Figure 3A). Statistical tests indicated significant differences in *Firmicutes* and *Proteobacteria* between the cancer group and the non-cancerous groups. At the species level, a total of 1,685 species were identified in the BE group, 223 in the EC group, 410 in the CIN group, and 709 in the CC group. *Lactobacillus iners* predominated in the non-cancerous groups, while *Klebsiella pneumoniae* predominated in the cancer group (Figure 3B). *Lactobacillus iners* was identified as a predominant bacterium associated with benign endometrial lesions and cervical cancer precursor lesions. Similarly, *Klebsiella pneumoniae* was observed as a dominant bacterium in both endometrial and cervical cancers.

Furthermore, different reproductive tract microbiota communities are distinguished by the varying dominance of *Lactobacillus*, resulting in distinct vaginal community state types (CSTs).[14] The cervicovaginal microbiome has been shown to belong to one of the CSTs.[25] Community-state types (CSTs) were assessed and quantified for each subject at the species level. We observed as the severity of the disease increased, indicating a decline in vaginal immunity, the proportion of CST-III decreased, while CST-IV, which is indicative of cervical canal dysbiosis, increased (Figure 3C).

Potential Microbial Biomarkers of the Cervical Canal in EC and CC

Having established the structural and compositional shifts, we next sought to identify stage-specific microbial signatures with diagnostic potential. To elucidate the vaginal microbial potential biomarkers distinguishing between BE and EC and between CIN and CC, two analytical approaches were employed. The results of the two methods intersect with each other to make them more representative. LEfSe analysis identified 37 microbial species as potential biomarkers in the endometrial group and 20 in the cervical cancer group. Specifically, *Lactobacillus iners* was identified as a potential biomarker common to the non-cancerous groups (LDA score > 4), whereas *Pasteurella multocida, Staphylococcus haemolyticus*, and *Staphylococcus aureus* were identified as potential biomarkers common to the cancer group (LDA score > 4) (Figure 4A). Volcano plot using limma analysis indicated 54 microbial species as potential biomarkers in the endometrial group and 130 in the cervical cancer group. Among the top 10 potential biomarkers ranked by limma analysis, *Lactobacillus iners* was identified as a potential biomarker common to the non-cancerous groups, whereas *Pasteurella multocida* was identified as a potential biomarker common to the cancer group (Figure 4B). The intersection of results from both methods identified eight, nineteen, four, and ten stringent potential biomarkers for the BE, EC, CIN, and CC groups, respectively (Figure 4C). *Lactobacillus iners* was identified as the stringent potential biomarker common to the non-cancerous groups, whereas *Pasteurella multocida, Pseudomonas putida, Ralstonia solanacearum, Burkholderia pseudomallei, Neisseria gonorrhoeae, Paracoccus mutanolyticus, Staphylococcus aureus*, and *Staphylococcus haemolyticus* were identified as stringent potential biomarkers common to the cancer group.

Interactions Among Differential Cervical Canal Microbial Biomarkers

To translate biomarker discovery into mechanistic insights, we investigated ecological relationships among differentially abundant species. Spearman correlation coefficients were computed to assess correlations among potential microbial biomarkers identified by LEfSe and limma, which were subsequently used to construct correlation heatmaps. The Spearman correlation results indicate that significant positive correlations predominantly exist among differential microbes across the four groups (Supplementary 2A–D). In the non-cancerous groups, *Lactobacillus iners* exhibited significant positive correlations with *Streptococcus cristatus, Lactobacillus reuteri*, and *Streptococcus sp. oral taxon 431* (Supplementary 2A and C). Conversely, in the cancer group, *Pasteurella multocida* showed significant positive correlations with *Burkholderia pseudomallei, Neisseria gonorrhoeae*, and *Staphylococcus haemolyticus*, while *Pseudomonas putida* showed significant positive correlations with *Neisseria gonorrhoeae*, and *Burkholderia pseudomallei* showed significant positive correlations with *Staphylococcus haemolyticus* (Supplementary 2B and D). Linear regression analyses were then conducted between potential biomarkers from the non-cancer and cancer groups. The results demonstrate a significant negative correlation between the non-cancer biomarker

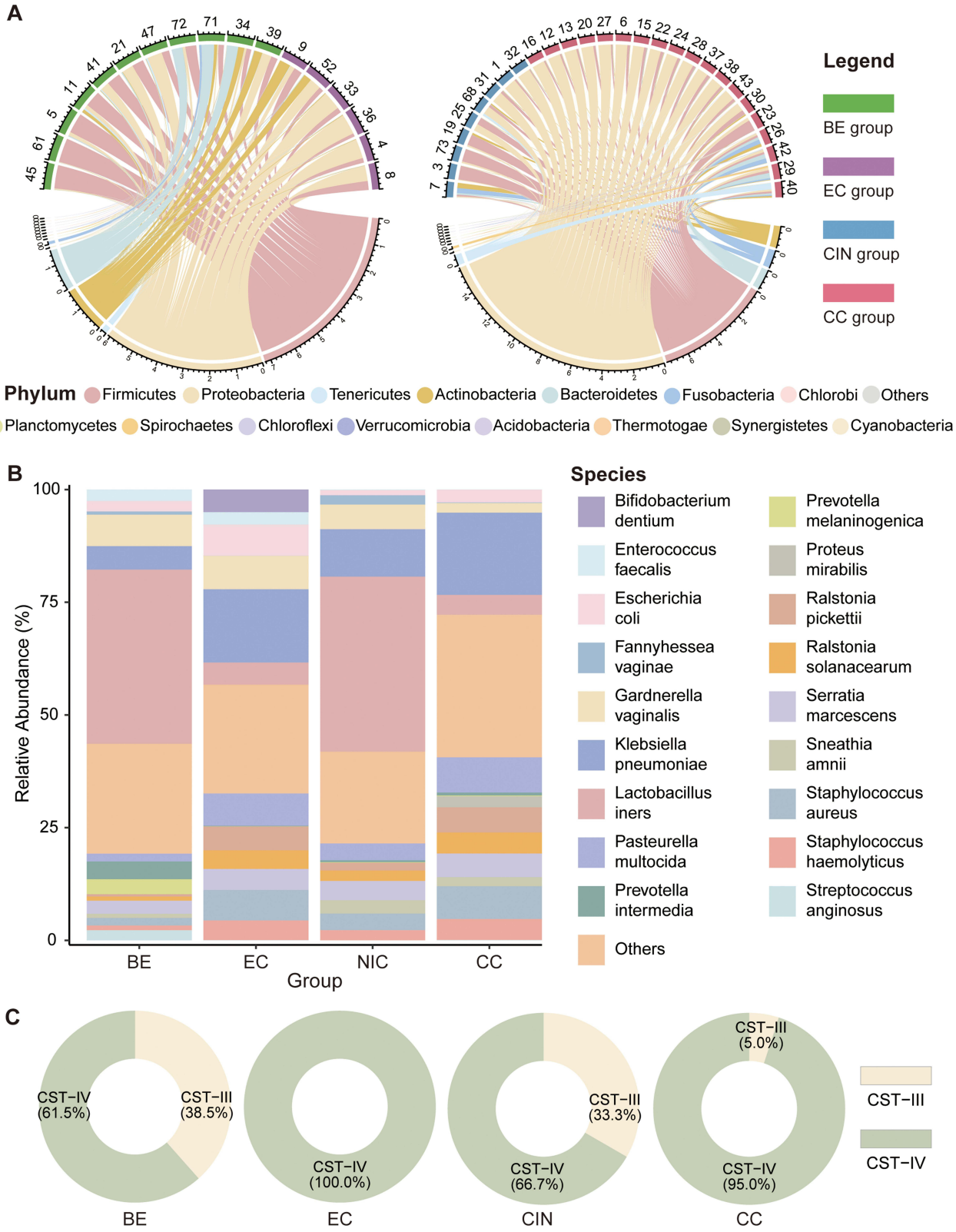

Figure 3 The relative abundances of taxonomy at phylum level and species level. (**A**) Microbial community composition at the phylum level of all samples. (**B**) Average relative abundance of microbial community composition at the level of species in the four groups. (**C**) Proportion of different community-state types in samples of four groups.

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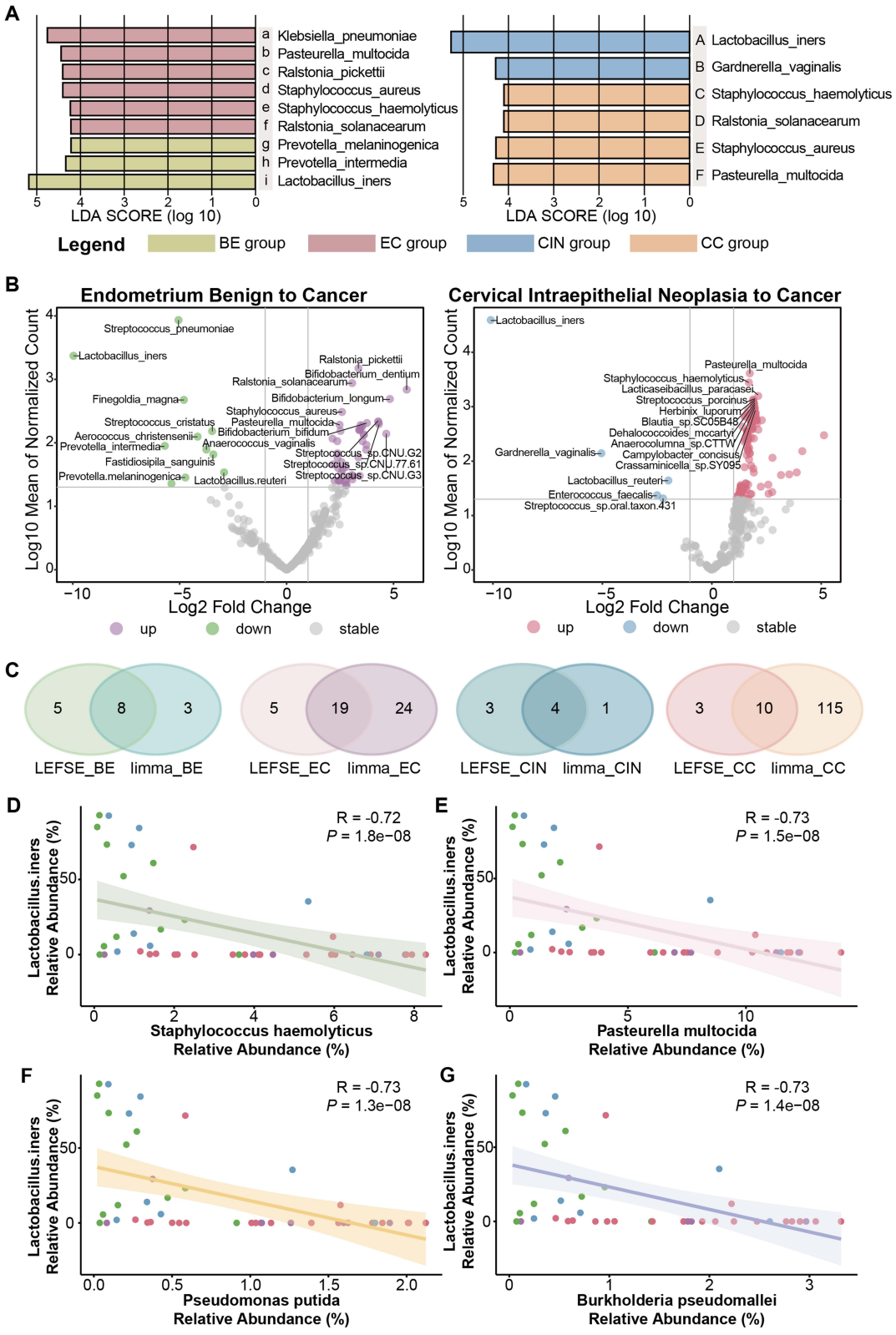

Figure 4 Analysis of biomarkers at species level. (**A**) LEfSe analysis of taxonomy with significant differences in abundance among groups. Histogram of LDA values, showing biomarkers with statistical differences between two groups (LDA > 4), and the length of the histogram represents the influence of the species with significant difference. (**B**) Differential analysis of abundance at the species level. The analysis of the volcano map showed the enrichment of bacteria at the species level in each group (**C**) Venn diagram was used to screen for common results between LEfSe analysis and analysis of the volcano map. Linear regression analyses were then conducted between potential biomarkers from the non-cancer and cancer groups. The results demonstrate a significant negative correlation between the non-cancer biomarker *Lactobacillus iners* and the cancer group biomarkers *Staphylococcus haemolyticus, Pasteurella multocida, Pseudomonas putida*, and *Burkholderia pseudomallei* (**D**–**G**).

Lactobacillus iners and the cancer group biomarkers *Staphylococcus haemolyticus, Pasteurella multocida, Pseudomonas putida*, and *Burkholderia pseudomallei* (Figure 4D–G).

Discussion

In this study, we investigated the cervical canal microbiota across four groups: BE, EC, CIN, and CC. We further explored the interactions and dynamics among microbiota communities. By comparing gynecological cancers with pre-cancerous lesions and benign tumors, we identified disturbances in the cervical canal microbiota associated with cancer. Our findings indicated that the EC group exhibited significantly different diversity compared to the BE group, and the CC group demonstrated significant differences from the CIN group. Alterations in the composition and co-occurrence networks of the cervical canal microbiota were observed. Differential analysis demonstrated that *Lactobacillus iners* was enriched in the BE and CIN groups, while *Pasteurella multocida, Pseudomonas putida, Burkholderia pseudomallei, Ralstonia solanacearum, Neisseria gonorrhoeae, Paracoccus mutanolyticus, Staphylococcus aureus*, and *Staphylococcus haemolyticus* were enriched in the EC and CC groups.

The lower reproductive tract microbiota symbiosis network is essential for understanding complex systems and ecological transitions.[26] Previous studies have demonstrated that, compared to healthy women, patients with uterine fibroids (benign tumors of the endometrial type) have microbiota networks with reduced connectivity and complexity,[27] indicating that the vaginal microbiota in patients with uterine fibroids may be less stable Similarly, under the same network parameters, we observed that the cancer groups (EC and CC) exhibited reductions in nodes, edges, modularity, and network characteristics to varying degrees compared to pre-cancerous lesions and benign tumors. The concurrent loss suggests a fundamental transition from stable, compartmentalized microbial ecosystems in pre-malignant states to depauperate, disorganized assemblages in invasive cancers. These ecological shifts in the microbiota could facilitate personalized diagnostic approaches in the future.[28] This also indicates that, a significant reduction in cervical canal microbiota interactions and stability in the cancer groups, potentially making them more susceptible to disturbances from external microorganisms, which could lead to vaginitis and disease.

Our multi-dimensional analysis revealed significant architectural reorganization of cervical canal microbiota across disease progression. Although the anatomical positions of the vagina and cervical canal are different, they are mainly composed of *Lactobacillus*.[29] Previous studies have demonstrated that the Shannon index does not significantly differ between EC and benign lesions,[30] and there is no notable difference in the Shannon or Simpson indices between cervical cancer and healthy controls,[31] which is consistent with our findings. However, we observed significant differences in the Chao1 index for EC and the Pielou index for CC. This suggests that malignancy progression may preferentially impact rare taxa distribution rather than overall species richness. Additionally, principal coordinate analysis and Adonis tests further confirmed significant differences in microbial diversity between groups, indicating substantial changes in vaginal microbiota structure under cancer conditions.[32]

Maintaining a balanced microbial environment is essential for reproductive tract health.[33] The dominance of *Lactobacillus iners* in the cervical canal microbiota generally signifies reduced vaginal immunity (CST-III). Dominance by strict anaerobes signifies dysbiosis (CST-IV).[14] The substantial changes observed at the phylum level between groups may reflect the critical role of microbial composition in disease progression. Species-level analysis further identified shifts in dominant bacterial populations associated with different disease states, with *Lactobacillus iners* predominating in pre-cancerous and benign tumor groups, and *Klebsiella pneumoniae* predominating in cancer groups. Typing of the cervical canal communities demonstrated an association between EC, CC, and cervical canal dysbiosis.[34,35] In conclusion, these findings enhance our understanding of the role of microbiota in cancer development and the potential use of biomarkers, thereby aiding in the assessment of key microorganisms involved in cervical canal dysbiosis.

Early diagnosis of cancer is crucial for improving patient survival.[36] Based on these findings, we further analyzed potential biomarkers in the cervical canal microbiota across different stages. The integrative analysis (LEfSe and limma) identified specific microbial populations associated with various cancer states. These findings establish a critical foundation for developing microbiota-based precision diagnostics. Consistent with previous studies, we observed an enrichment of *Lactobacillus iners* in pre-cancerous and benign tumor groups, as well as opportunistic pathogens in cancer groups.[32,37] This suggests that the cervical canal ecosystem is disrupted, resulting in excessive growth of pathogens and complex infections.[38] The identification of these biomarkers not only aids in distinguishing between disease states but also provides new biological insights for early diagnosis, treatment strategies, and screening of high-risk populations. Particularly, the *Lactobacillus iners*/ *Klebsiella pneumoniae* abundance ratio may serve as a promising diagnostic biomarker.

The vaginal microbiota exhibits high dynamism.[39] Our Spearman correlation and linear regression analyses revealed that, during the progression from pre-cancerous or benign tumor groups to cancer, the *Lactobacillus iners*-dominated vaginal microbiota is gradually replaced by anaerobic microbiota communities dominated by *Staphylococcus haemolyticus, Pasteurella multocida, Pseudomonas putida*, and *Burkholderia pseudomallei*. Studies have shown that various *Lactobacillus* species affect the stability of the vaginal microbiota differently, with *Lactobacillus iners* being less effective in maintaining stability, thereby contributing to dysbiosis.[40] This may be attributed to the production of L-lactic acid by *Lactobacillus iners*, which is insufficient to inhibit the progression of pathogen infections during cancer. The limited metabolic capacity and dependence on host nutrients of *Lactobacillus iners* facilitate its replacement by opportunistic pathogens.[41] Research suggests that Lactobacilli possess protective anti-tumor effects on the cervical epithelium.[42] Therefore, early regulation and restoration of a *Lactobacillus*-dominant lower reproductive tract microbiota may help mitigate cancer progression and improve prognosis.[43] Currently, Lactobacilli are widely utilized as alternatives for preventing chronic vaginitis and restoring the vaginal ecosystem, supplementing conventional antimicrobial treatments for vaginal pathogens.[44] Moreover, the effectiveness of long-term *Lactobacillus* administration in prevention has been well established.[38]

The translation from microbial biomarker discovery to clinical deployment necessitates addressing the ethical and governance complexities intrinsic to personalized medicine. While our identified *Lactobacillus iners, Klebsiella pneumoniae* and other opportunistic pathogen exhibit diagnostic utility and complementary therapies, its integration into precision oncology pipelines raises critical questions regarding equitable accessibility and algorithmic accountability.[45] Emerging evidence demonstrates that microbiota-modulating interventions have the potential to redefine cancer immunotherapy paradigms.[46] However, the clinical translation of such approaches must confront multifaceted challenges: social acceptability, regulatory harmonization.[47] The successful translation of our findings requires reimagining healthcare delivery through the lens of ecological precision medicine–where microbial network restoration becomes as fundamental as tumor genomic profiling in cancer management. This paradigm shift demands collaborative governance models engaging policymakers, bioethicists and community representatives to co-design guidelines balancing innovation with equity.

Conclusion

Our metagenomic analysis provides evidence of profound ecological restructuring in the cervical canal microbiota among patients with endometrial and cervical cancers. The progressive collapse of microbial network complexity—marked by reduced modularity and connectivity—correlates with disease progression from BE/CIN to EC/CC. Critically, we identify two microbial hallmarks: *Lactobacillus iners* depletion and pathogen consortium emergence. By bridging ecological theory with precision oncology, these findings pioneer a microbiome-informed paradigm for gynecologic cancer diagnosis and complementary therapies. Nonetheless, the mechanisms through which pathogenic bacteria and microbial ecological imbalances influence gynecological tumors necessitate further investigation.

Ethical Approval

The study was approved by the Ethics Committee of Hunan Cancer Hospital (KYJJ-2023-025) and followed the Declaration of Helsinki on Biology for Human Trials. Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Brooks RA, Fleming GF, Lastra RR, et al. Current recommendations and recent progress in endometrial cancer. *CA Cancer J Clin*. 2019;69(4):258–279. doi:10.3322/caac.21561
2. Zheng RS, Chen R, Han BF, et al. Cancer incidence and mortality in China, 2022. *Zhonghua Zhong Liu Za Zhi*. 2024;46(3):221–231. doi:10.3760/cma.j.cn112152-20240119-00035
3. Abu-Rustum NR, Yashar CM, Bradley K, et al. NCCN Guidelines® Insights: Uterine Neoplasms, Version 3.2021. *J Natl Compr Canc Netw*. 2021;19(8):888–895. doi:10.6004/jnccn.2021.0038
4. Crosbie EJ, Kitson SJ, McAlpine JN, Mukhopadhyay A, Powell ME, Singh N. Endometrial cancer. *Lancet*. 2022;399(10333):1412–1428. doi:10.1016/S0140-6736(22)00323-3
5. Bautista OM, Saah A, Muñoz N. Re: longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. *J Natl Cancer Inst*. 2011;103(2):158;authorreply158–159. doi:10.1093/jnci/djq468
6. Makker V, MacKay H, Ray-Coquard I, et al. Endometrial cancer. *Nat Rev Dis Primers*. 2021;7(1):88. doi:10.1038/s41572-021-00324-8
7. Kaptilnyy VA, Александрович КВ, Efimova VA, Андреевна ЕВ, Lazarenko AN, Николаевна ЛА. Possibilities and prospects of targeted therapy for persistent human papillomavirus infection. *VFSnegirev Arch Obst Gynecol*. 2023;10(1):13–24. doi:10.17816/2313-8726-2023-10-1-13-24
8. Marima R, Hull R, Mbeje M, et al. Role of precision oncology in type II endometrial and prostate cancers in the African Population: global cancer genomics disparities. *Int J Mol Sci*. 2022;23(2):628. doi:10.3390/ijms23020628
9. Shin SH, Bode AM, Dong Z. Addressing the challenges of applying precision oncology. *Npj Precision Onc*. 2017;1(1):1–10. doi:10.1038/s41698-017-0032-z
10. Piergentili R, Basile G, Nocella C, et al. Using ncRNAs as tools in cancer diagnosis and treatment—the way towards personalized medicine to improve patients' health. *Int J Mol Sci*. 2022;23(16):9353. doi:10.3390/ijms23169353
11. Elmore LW, Greer SF, Daniels EC, et al. Blueprint for cancer research: critical gaps and opportunities. *CA Cancer J Clin*. 2021;71(2):107–139. doi:10.3322/caac.21652
12. Oyovwi MO, Ben-Azu B, Babawale KH. Therapeutic potential of microbiome modulation in reproductive cancers. *Med Oncol*. 2025;42(5):152. doi:10.1007/s12032-025-02708-2
13. Zhu B, Tao Z, Edupuganti L, Serrano MG, Buck GA. Roles of the microbiota of the female reproductive tract in gynecological and reproductive health. *Microbiol Mol Biol Rev*. 2022;86(4):e0018121. doi:10.1128/mmbr.00181-21
14. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A*. 2011;108(Suppl 1):4680–4687. doi:10.1073/pnas.1002611107
15. Gupta S, Kakkar V, Bhushan I. Crosstalk between vaginal microbiome and female health: a review. *Microb pathog*. 2019;136:103696. doi:10.1016/j.micpath.2019.103696
16. O'Hanlon DE, Moench TR, Cone RA. Vaginal pH and microbicidal lactic acid when lactobacilli dominate the microbiota. *PLoS One*. 2013;8(11):e80074. doi:10.1371/journal.pone.0080074
17. Kimberlin DF, Andrews WW. Bacterial vaginosis: association with adverse pregnancy outcome. *Semin Perinatol*. 1998;22(4):242–250. doi:10.1016/s0146-0005(98)80012-8
18. Shroff S. Infectious vaginitis, cervicitis, and pelvic inflammatory disease. *Med Clin North Am*. 2023;107(2):299–315. doi:10.1016/j.mcna.2022.10.009
19. Yl H, Xy L, L J, Zn X, C J, Y J. Vaginal microbiome dysbiosis as a novel noninvasive biomarker for detection of chronic endometritis in infertile women. *Int J Gynaecol Obst*. 2024;167(3). doi:10.1002/ijgo.15779
20. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*. 2018;34(17):i884–i890. doi:10.1093/bioinformatics/bty560
21. Xu H, Luo X, Qian J, et al. FastUniq: a fast de novo duplicates removal tool for paired short reads. *PLoS One*. 2012;7(12):e52249. doi:10.1371/journal.pone.0052249
22. Langdon WB. Performance of genetic programming optimised Bowtie2 on genome comparison and analytic testing (GCAT) benchmarks. *BioData Min*. 2015;8(1):1. doi:10.1186/s13040-014-0034-0
23. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Bio*. 2019;20(1):257. doi:10.1186/s13059-019-1891-0
24. Lu J, Breitwieser FP, Thielen P, Salzberg SL. Bracken: estimating species abundance in metagenomics data. *PeerJ Comput Sci*. 2017;3:e104. doi:10.7717/peerj-cs.104
25. Klein C, Kahesa C, Mwaiselage J, West JT, Wood C, Angeletti PC. How the cervical microbiota contributes to cervical cancer risk in Sub-Saharan Africa. *Front Cell Infect Microbiol*. 2020;10:10. doi:10.3389/fcimb.2020.00023
26. Greenbaum S, Greenbaum G, Moran-Gilad J, Weintraub AY. Ecological dynamics of the vaginal microbiome in relation to health and disease. *Am J Obst Gynecol*. 2019;220(4):324–335. doi:10.1016/j.ajog.2018.11.1089
27. Mao X, Chen H, Peng X, Zhao X, Yu Z, Xu D. Dysbiosis of vaginal and cervical microbiome is associated with uterine fibroids. *Front Cell Infect Microbiol*. 2023;13:1196823. doi:10.3389/fcimb.2023.1196823
28. Li J, Li Y. Detect feature edges for diagnosis of bacterial vaginosis. *Peerj*. 2023;11:e14667. doi:10.7717/peerj.14667

29. Chen C, Song X, Wei W, et al. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nat Commun*. 2017;8(1):875. doi:10.1038/s41467-017-00901-0
30. Walsh DM, Hokenstad AN, Chen J, et al. Postmenopause as a key factor in the composition of the Endometrial Cancer Microbiome (ECbiome). *Sci Rep*. 2019;9(1):19213. doi:10.1038/s41598-019-55720-8
31. Mitra A, MacIntyre DA, Lee YS, et al. Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. *Sci Rep*. 2015;5(1):16865. doi:10.1038/srep16865
32. Walther-António MRS, Chen J, Multinu F, et al. Potential contribution of the uterine microbiome in the development of endometrial cancer. *Genome Med*. 2016;8(1):122. doi:10.1186/s13073-016-0368-y
33. Zhang M, Zhou Y, Yao S, et al. Effect of stress urinary incontinence on vaginal microbial communities. *BMC Microbiol*. 2024;24(1):112. doi:10.1186/s12866-024-03237-0
34. Brusselaers N, Shrestha S, van de Wijgert J, Verstraelen H. Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. *Am J Obst Gynecol*. 2019;221(1):9–18.e8. doi:10.1016/j.ajog.2018.12.011
35. Ilhan ZE, Łaniewski P, Thomas N, Roe DJ, Chase DM, Herbst-Kralovetz MM. Deciphering the complex interplay between microbiota, HPV, inflammation and cancer through cervicovaginal metabolic profiling. *EBioMedicine*. 2019;44:675–690. doi:10.1016/j.ebiom.2019.04.028
36. Han M, Wang N, Han W, Ban M, Sun T, Xu J. Vaginal and tumor microbiomes in gynecological cancer (Review). *Oncol Lett*. 2023;25(4):153. doi:10.3892/ol.2023.13739
37. Kang GU, Jung DR, Lee YH, et al. Potential association between vaginal microbiota and cervical carcinogenesis in Korean women: a cohort study. *Microorganisms*. 2021;9(2):294. doi:10.3390/microorganisms9020294
38. Chee WJY, Chew SY, Than LTL. Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal health. *Microb Cell Fact*. 2020;19(1):203. doi:10.1186/s12934-020-01464-4
39. Gajer P, Brotman RM, Bai G, et al. Temporal dynamics of the human vaginal microbiota. *Sci Transl Med*. 2012;4(132):132ra52. doi:10.1126/scitranslmed.3003605
40. Petrova MI, Reid G, Vaneechoutte M, Lebeer S. Lactobacillus iners: friend or Foe? *Trends Microbiol*. 2017;25(3):182–191. doi:10.1016/j.tim.2016.11.007
41. France MT, Mendes-Soares H, Forney LJ. Genomic comparisons of Lactobacillus crispatus and Lactobacillus iners reveal potential ecological drivers of community composition in the vagina. *Appl Environ Microbiol*. 2016;82(24):7063–7073. doi:10.1128/AEM.02385-16
42. Wang KD, Xu DJ, Wang BY, Yan DH, Lv Z, Su JR. Inhibitory effect of vaginal lactobacillus supernatants on cervical cancer cells. *Probiotics Antimicro Prot*. 2018;10(2):236–242. doi:10.1007/s12602-017-9339-x
43. Anahtar MN, Gootenberg DB, Mitchell CM, Kwon DS. Cervicovaginal microbiota and reproductive health: the virtue of simplicity. *Cell Host Microbe*. 2018;23(2):159–168. doi:10.1016/j.chom.2018.01.013
44. De Alberti D, Russo R, Terruzzi F, Nobile V, Ouwehand AC. Lactobacilli vaginal colonisation after oral consumption of Respecta(®) complex: a randomised controlled pilot study. *Arch Gynecol Obstet*. 2015;292(4):861–867. doi:10.1007/s00404-015-3711-4
45. Salari P, Larijani B. Ethical Issues Surrounding Personalized Medicine: a Literature Review. *Acta Med Iran*. 2017;55(3):209–217.
46. McKenzie ND, Hong H, Ahmad S, Holloway RW. The gut microbiome and cancer immunotherapeutics: a review of emerging data and implications for future gynecologic cancer research. *Crit Rev Oncol Hematol*. 2021;157:103165. doi:10.1016/j.critrevonc.2020.103165
47. Sharrer GT. Personalized medicine: ethical aspects. *Methods Mol Biol*. 2017;1606:37–50. doi:10.1007/978-1-4939-6990-6_3