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Altered microbial diversity and composition of multiple mucosal organs in cervical cancer patients

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Abstract

Objectives The aim of this study was to characterize the microbiome of multiple mucosal organs in cervical cancer (CC) patients.

Methods We collected oral, gut, urinary tract, and vaginal samples from enrolled study participants, as well as tumor tissue from CC patients. The microbiota of different mucosal organs was identified by 16S rDNA sequencing and correlated with clinical-pathological characteristics of cervical cancer cases.

Results Compared with controls, CC patients had reduced α-diversity of oral and gut microbiota ($p_{Oral\_Sob} < 0.001$, $p_{Oral\_Shannon} = 0.049$, $p_{Oral\_Simpson} = 0.013$ $p_{Fecal\_Sob} = 0.030$), although there was an opposite trend in the vaginal microbiota ($p_{Vaginal\_Pielou} = 0.028$, $p_{Vaginal\_Simpson} = 0.006$). There were also significant differences in the β-diversity of the microbiota at each site between cases and controls ($p_{Oral} = 0.002$, $p_{Fecal} = 0.037$, $p_{Urine} = 0.001$, $p_{Vaginal} = 0.001$). The uniformity of urine microbiota was lower in patients with cervical squamous cell carcinoma ($p_{Urine} = 0.036$) and lymph node metastasis ($p_{Urine\_Sob} = 0.027$, $p_{Urine\_Pielou} = 0.028$, $p_{Urine\_Simpson} = 0.021$, $p_{Urine\_Shannon} = 0.047$). The composition of bacteria in urine also varied among patients with different ages ($p = 0.002$), tumor stages ($p = 0.001$) and lymph node metastasis ($p = 0.002$). In CC cases, *Pseudomonas* were significantly enriched in the oral, gut, and urinary tract samples. In addition, *Gardnerella*, *Anaerococcus*, and *Prevotella* were biomarkers of urinary tract microbiota; *Abiotrophia* and *Lautropia* were obviously enriched in the oral microbiota. The microbiota of tumor tissue correlated with other mucosal organs (except the gut), with a shift in the microflora between mucosal organs and tumors.

Conclusions Our study not only revealed differences in the composition and diversity of the vaginal and gut microflora between CC cases and controls, but also showed dysbiosis of the oral cavity and urethra in cervical cancer cases.

Keywords Cervical cancer, 16S rDNA sequencing, Multi-organ microbiome, Dysbiosis

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Introduction

According to the GLOBOCAN report in 2020, cervical cancer (CC) remained the most common malignancy of the female reproductive tract globally [1]. High-risk human papillomavirus (HPV) infection has been implicated as the cause of cervical cancer in most cases [2]. In recent years, ecological disorders of the intestines and vagina have been shown to increase the risk of CC through inflammation as well as metabolic and immune alterations [3, 4], although there are few reports of the relationship between intra-tumoral and other organ microbiota with CC.

The human microbiome contains 100-fold more genetic diversity than the host genome and affects important physiological functions [5, 6]. The microbiome is now regarded as an important part of the tumor microenvironment, and the complex interactions with the host can affect tumor occurrence and progression locally [7]. The mucosal organs, such as the oral cavity, gastrointestinal tract (GIT), urinary tract (UT), and female reproductive tract (FRT), which have a huge mucosal surface area and are in contact with the external environment, are important sites for host-microbial interaction. Numerous studies have shown that dysregulation of the microbiome in various mucosal organs is related to the carcinogenesis of adjacent or distant organs [8–11]. The healthy FRT has a specific microbiota composed mainly of *Lactobacillus* species, which create an acidic environment for the vagina and to protect against opportunistic infections [12]. Vaginal dysbiosis may lead to CC by affecting immune regulation, mediating HPV infection, and inducing inflammation [13]. The GIT serves as the primary habitat of the human microflora, and changes in the gut microbiota can lead to disturbances in estrogen metabolism levels and play an active role in the development of CC [14].

The oral cavity, which has the second richest microbiome in the human body, has been implicated in oral squamous cell carcinoma and various cancers in distant organs [15, 16], but there are very limited studies on the oral microbiota relating to CC. Studies using technologies such as high-throughput sequencing have demonstrated the existence of a specific microbiota in the UT, challenging the long-held theory that the proximal urethra is a sterile environment [17]. The microbiota present in the UT has been identified as a pathogenic factor or cofactor in the development of genitourinary malignancies [18]. Studies have indicated the interactions between the FRT microbiome and the distal mucosal organs (oral and UT) [14]; therefore, we hypothesized that dysbacteriosis of the oral and urine flora may increase the risk of CC.

In this study, we explored the relationship between the microbiota of distal mucosal organs (oral and UT) and CC and the changes in the composition and diversity of the microbiome in multiple mucosal organs of CC patients, as well as the correlation and metastasis between individual mucosal organs and microorganisms within the tumor. This study provides a foundation for further exploration of the role of the microbiota in CC risk and treatment.

Methods

Study participants and ethical statement

Patients with CC admitted to the Third Affiliated Hospital of Kunming Medical University between December 2021 and July 2022 were selected. Patients who met the following criteria were enrolled: (i) All CC patients were pathologically diagnosed as squamous cell carcinoma or adenocarcinoma. (ii) Age: 18–75 years. (iii) At the time of initial diagnosis of CC, there were no tumors in other sites and patients did not receive antineoplastic therapy. (iv) No antibiotics, probiotics, and other drugs used within one month prior to biological sample collection. The exclusion criteria were as follows: (i) Pathological diagnosis of cervical sarcoma, neuroendocrine carcinoma, peripheral schwannoma, or malignant melanoma. (ii) Vaginal lavage within 1 week. (iii) Had sex within 48 h. Tumor-free volunteers were recruited by the Physical Examination Center of the Third Affiliated Hospital of Kunming Medical University as controls. The inclusion and exclusion criteria for controls were the same as those for the CC cases, except for any cancer diagnosis. All clinical data, including age, ethnicity, number of pregnancies and births, cancer stage, and tumor size were collected through hospital visit records and inpatient records. CC cases included in the study were staged according to the Federation of Gynecology and Obstetrics (FIGO) 2018 staging system. Maximum tumor size and lymph node metastasis were determined by computed tomography and magnetic resonance imaging.

Sample collection and DNA extraction

During gynecological examination, clean biopsy forceps were used to remove CC tissue, and disposable sterile cotton swabs were used to collect secretions from the posterior fornix of the vagina. On the morning after gynecological examination, fecal and midstream urine samples were collected into two 50-ml clean centrifuge tubes. For each subject, oral specimens were collected by repeated wiping of the cheek and sides of the tongue with disposable sterile cotton swabs. All 404 samples were snap-frozen in liquid nitrogen and transferred to a -80 °C freezer within 1 h after collection. Microbial DNA was extracted from all biological samples using the HiPure

Stool DNA Kits (Magen, Guangzhou, China) according to manufacturer's protocols.

16S rDNA gene sequencing

The 16S rDNA V3–V4 region of the ribosomal RNA gene were amplified by PCR using specific primers (341F-CCT AYGGGRBGCASCAG, and 806R-GGACTACNNGGG TATCTAAT) with barcodes and reagents purchased from New England Biolabs, USA. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using the ABI Step One Plus Real-Time PCR System (Life Technologies, Foster City, CA, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced (PE250) on an Illumina platform according to standard protocols at Guangzhou Genedenovo Biotechnology Co., Ltd (Guangzhou, China). PCR amplification failed in three urethral specimens and two vaginal samples from patients; therefore, 399 samples were successfully sequenced.

Sequence data processing

Raw reads were filtered to get high-quality clean reads according to the following rules using FASTP (version 0.18.0): (1) Removing reads containing more than 10% of unknown nucleotides (N); (2) Removing reads containing less than 50% of bases with quality (Q-value) > 20; (3) Removing adapter contamination. Pared reads were overlapped as raw tags using FLASH (version 1.2.11) with a minimum overlap of 10 bp and mismatch error rates of 2%. Noisy sequences were filtered to obtain high-quality clean sequences as following conditions: (1) Break raw tags from the first low quality base site where the number of bases in the continuous low quality value (the default quality threshold is ≤ 3) reaches the set length (the default length is 3 bp); (2) Filter tags whose continuous high-quality base length is less than 75% of the tag length. The clean tags were clustered into operational taxonomic units (OTUs) of $\geq 97\%$ similarity using UPARSE (version 9.2.64) pipeline. The representative OUT sequences were classified into organisms with a naive Bayesian model according to the SILVA database (version 138.1).

Data analysis

The relative abundance of microbial taxa at the genus level was compared between the case and control groups. The abundance statistics of each taxonomy were visualized using Krona (version 2.6). The stacked bar plot of the community composition was visualized in the R progect ggplot2 package (version 2.2.1). Sob index, Pielou index, Shannon index, and Simpson index were used as α-diversity indexes, and Wilcoxon tests were used to evaluate the significance. The β-diversity index was used to compare microbiome structure between groups. The principal coordinate analysis (PCoA) and Adonis tests were performed based on Bray-Curtis distance to assess β-diversity of microbial composition. The linear discriminant analysis (LDA) effect size (LEfSe) was used to assess the specific main microbiome in each group and develop biomarkers. In order to achieve high specificity, an LDA score > 3.5 was considered the cut-off value for biomarker screening. We used a permutation test based on the Bray-Curtis distance to examine the correlation between the test organ, and the taxon-specific Euclidean distance was used to determine the bacteria shared by the two organs [19]. Based on the presence and absence of sample abundance, the values of species in each sample were set to 1 and 0, and the Euclidean distance matrix was calculated for each species. The permutation test of the distance matrix was carried out according to the grouping information, and the p-value of the difference between the two groups was obtained. $p < 0.05$ was set as the threshold for statistical significance.

Results

Characteristics of subjects

As shown in Supplementary Tables 1, 72 CC patients and 11 healthy women as controls were included in this study. The mean age of CC and healthy controls was 53.03 and 45.64 years, respectively. Compared with controls, the ethnic composition of the CC patient group was more complex, and more pregnancies and births were recorded. In terms of HPV infection status, approximately one-third of the patients (24 of 72) were infected with HPV-16, followed by other genotypes of HPV (19.4%) and HPV-18 (5.6%). Among the CC patients, 27.8% were HPV-negative, and 26.4% had unknown HPV status. Clinical data from 72 CC patients showed that around a half of patients (40 of 72) were in stage III, and most had cervical squamous cell carcinoma (87.5%). Approximately 62.5% of patients had tumors with a diameter ≥ 4 cm, and about 52.8% had lymph node metastases.

Differential microbial diversity among multiple mucosal organ microbiotas

Both in CC patients and in healthy controls, we detected differences in the microbial composition and relative abundance of individual organs at the genus level (Fig. 1A-D). The microbial diversity of the vagina was significantly lower than that of other mucosal organs (Fig. 1E-F). Both the healthy controls and CC patients showed significant differences in β-diversity of the microbial composition in multiple mucosal organs. (Fig. 1G-H).

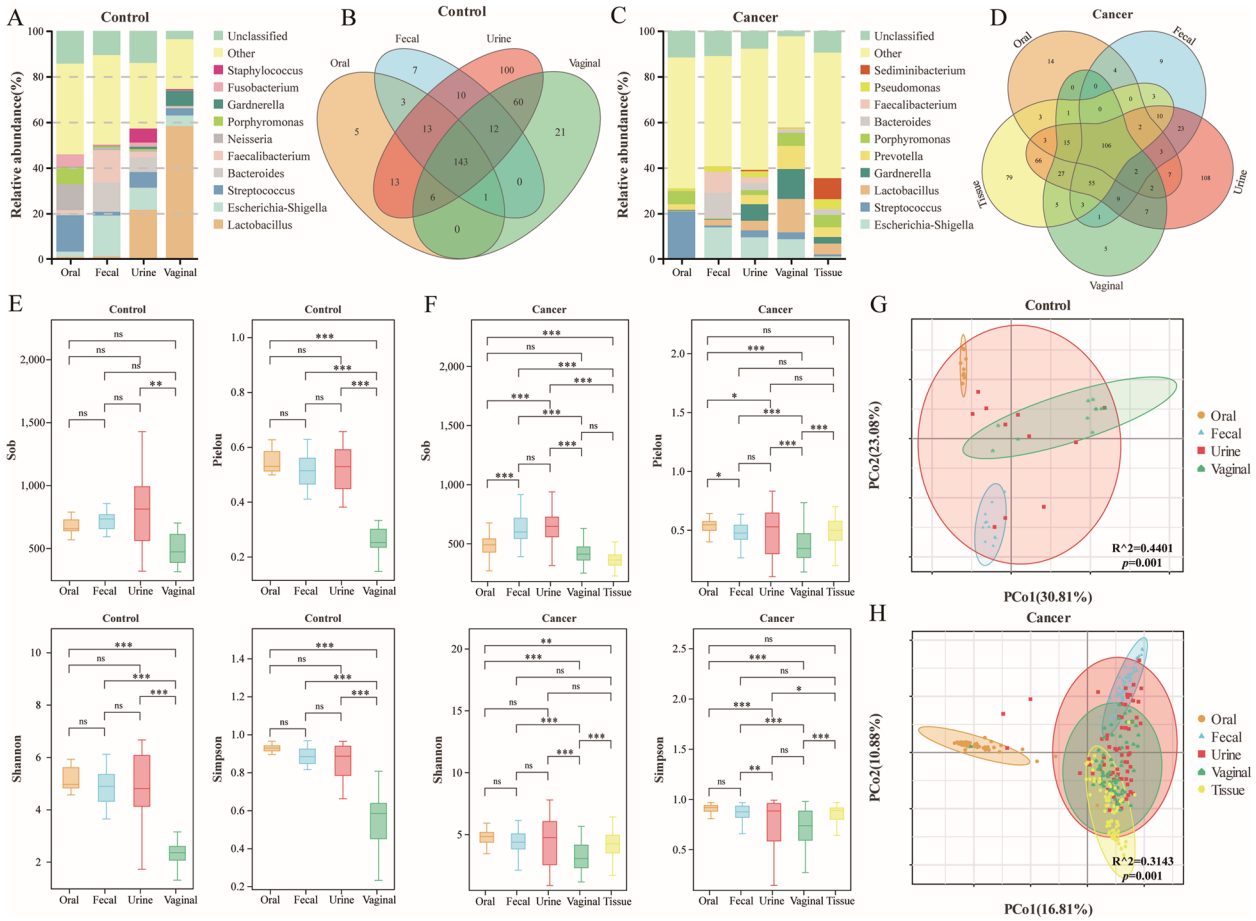

Fig. 1 Differences in species composition and biodiversity among multiple mucosal organ microbiotas

Multi-organ microbiome characterization in healthy control subjects and cervical cancer cases

In CC cases, the relative abundances of *Veillonella* ($p = 0.025$) and *Capnocytophaga* ($p = 0.020$) in the oral cavity were increased compared to healthy controls (Fig. 2A). The gut microbiota was dominated by *Escherichia-Shigella*, *Bacteroides* and *Faecalibacterium*. The relative abundances of *Citrobacter* ($p = 0.013$) and *Prevotella_9* ($p = 0.004$) showed an increasing trend in CC patients (Fig. 2B). In the urethra, *Lactobacillus* was predominant in healthy controls, whereas its abundance was decreased in CC patients, with an increase in *Enterococcus* ($p = 0.006$), *Gardnerella* ($p = 0.005$), *Prevotella* ($p < 0.001$) and *Anaerococcus* ($p < 0.001$) (Fig. 2C). The vaginal microbiota of healthy controls was particularly relevant with *Lactobacillus* ($p = 0.004$), while *Prevotella* ($p < 0.001$), *Porphyromonas* ($p < 0.001$), *Anaerococcus* ($p < 0.001$) and *Peptoniphilus* ($p < 0.001$) were more relevant in the CC patients (Fig. 2D). *Ralstonia* and *Sediminibacterium* were dominant bacteria in CC tissues(Fig. 2E).

We next analyzed dysbiosis of the microbiota colonizing various organs in CC patients and healthy controls. The species richness of the oral and fecal microbiota of CC patients was lower than that of the healthy control group ($p_{Oral\_Sob} < 0.001$, $p_{Fecal\_Sob} = 0.030$), although there was no significant difference in the uniformity ($p_{Oral\_Pielou} = 0.550$, $p_{Fecal\_Pielou} = 0.149$) (Fig. 3A, B). However, there was no significant difference in α-diversity between the CC and healthy control groups ($p_{Urine\_Sob} = 0.119$, $p_{Urine\_Pielou} = 0.911$, $p_{Urine\_Simpson} = 0.539$, $p_{Urine\_Shannon} = 0.889$) in the UT microbiome (Fig. 3C). We also found that the uniformity of the vaginal microbiota in CC patients was higher than that of controls ($p_{Vaginal\_Pielou} = 0.028$), while species richness decreased, although this did not reach the level of statistical significance ($p_{Vaginal\_Sob} = 0.154$) (Fig. 3D). There were significant differences in the microbial composition of the oral, gut, urethra, and vaginal between healthy controls and patients ($p_{Oral} = 0.002$, $p_{Fecal} = 0.037$, $p_{Urine} = 0.001$, $p_{Vaginal} = 0.001$) (Fig. 3E-H).

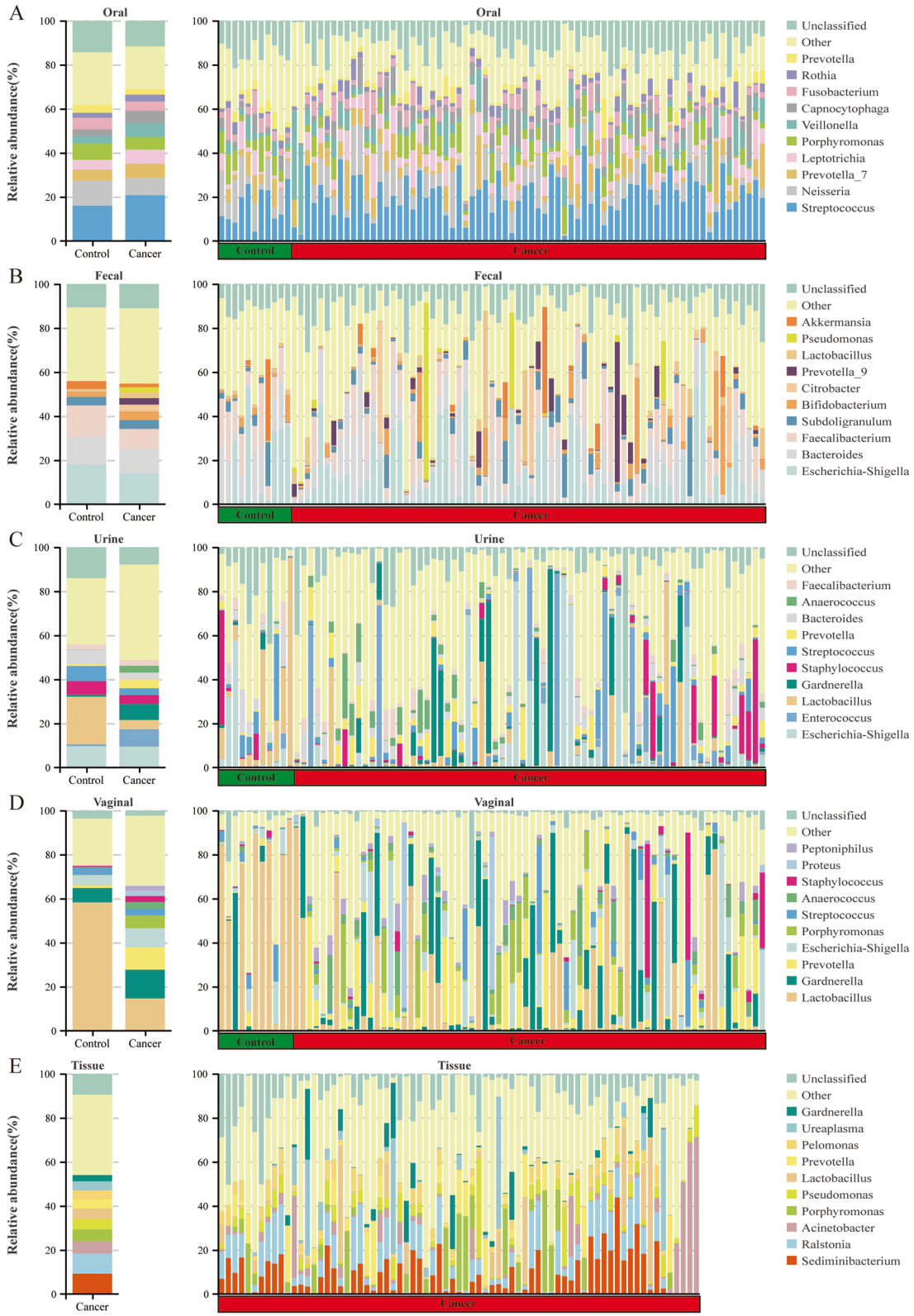

Fig. 2 Genus-level differences identified in cervical cancer patients versus healthy controls

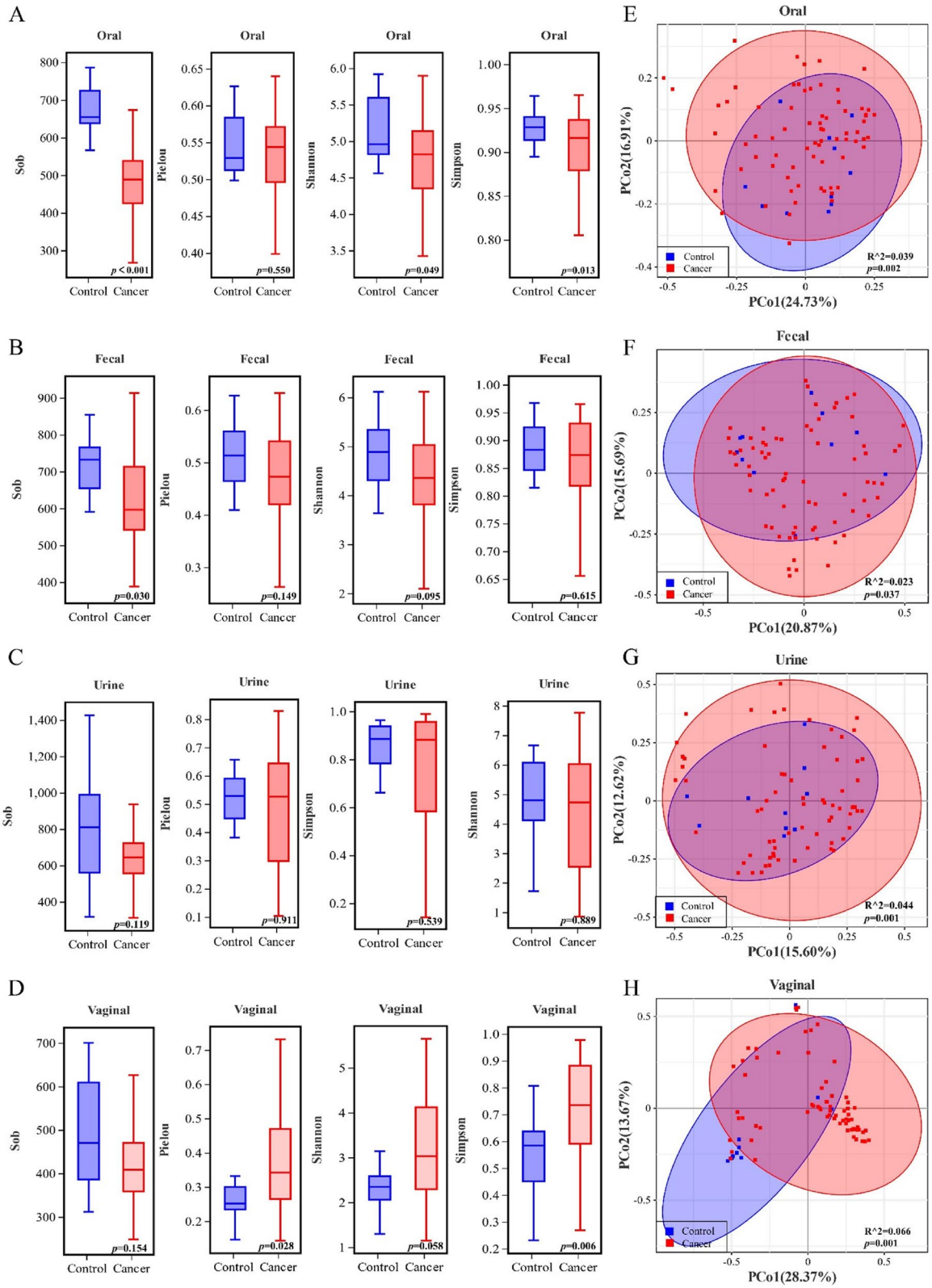

Fig. 3 Microbial characteristics in healthy control subjects and cervical cancer cases

Characteristics of multi-organ microbiome in CC patients with different clinical-pathological features

As shown in Supplementary Fig. 1 and Supplementary Table 2, the uniformity of the vaginal and tumor tissue microbiomes was increased in older women (≥ 50 years) ($p_{Vaginal\_Pielou} = 0.043$, $p_{Tumor\_Pielou} = 0.029$). There were statistically significant differences in the urethral, vaginal, and tumor tissue bacterial community compositions between younger and older women ($p_{Urine} = 0.002$, $p_{Vaginal} = 0.001$, $p_{Tumor} = 0.002$). The uniformity of the cervical adenocarcinoma microbiome was greater than that of cervical squamous cell carcinoma ($p_{Urine} = 0.036$) in urine samples. In terms of cancer stages, there were differences in α-diversities ($p_{Urine\_Sob} = 0.021$, $p_{Vaginal\_Pielou} = 0.016$, $p_{Vaginal\_Simpson} = 0.008$, $p_{Vaginal\_Shannon} = 0.017$) and β-diversity ($p_{Urine} = 0.001$, $p_{Vaginal} = 0.002$) of the urine and vaginal sample microflora.The α-diversities ($p_{Urine\_Sob} = 0.027$, $p_{Urine\_Pielou} = 0.028$, $p_{Urine\_Simpson} = 0.021$, $p_{Urine\_Shannon} = 0.047$) of the urethra microbiota in patients with lymph node metastases was lower than in patients without lymph node metastases, with significant differences in the β-diversities of the gut and urethral microbiota ($p_{Fecal} = 0.033$, $p_{Urine} = 0.002$) between the two groups.

Differentially expressed microbial biomarkers in multi-organ microbiomes

We then performed LEfSe analysis to identify marker genera at different sites that distinguish CC patients from healthy controls ($p < 0.05$, LDA score > 3.5). *Pseudomonas*, *Abiotrophia*, and *Lautropia* were significantly enriched in CC oral samples (Fig. 4A, Supplementary Fig. 2A). Five marker genera, *Citrobacter*, *Prevotella_9*, *Romboutsia*, *Lactobacillus*, and *Pseudomonas* family were identified for the CC group (Fig. 4B, Supplementary Fig. 2B). In the analysis of the urethral microbiota, *Gardnerella*, *Anaerococcus*, *Prevotella*, *Pseudomonas*, and *Peptoniphilus* were identified as significantly enriched in CC samples (Fig. 4C, Supplementary Fig. 2C). *Prevotella*, *Porphyromonas*, *Gardnerella*, *Proteus*, and *Anaerococcus* were marker genus for the vaginal microbiome of CC patients (Fig. 4D, Supplementary Fig. 2D).

Correlations between the microbiomes of various organs

We assessed the correlation of microbiomes in different organs in patients with CC. We identified a significant correlation between the microbiomes of tumor tissue in CC patients and other organs, except the gut (Supplementary Table 3). Analysis at the genus level revealed several bacterial genera that were significantly shared between the microbiomes of the tumor tissue and other organs (Supplementary Table 4).

Discussion

In this study, we discovered that the α-diversity of oral and gut microbiota in CC patients was lower compared to healthy individuals, while the opposite trend was found in vaginal microflora. There were differences in the microbial composition of multiple mucosal organs between CC patients and healthy women. LEfSe analysis showed that *Prevotella* is more abundant in the vaginal, gut, and urethral microbiome of CC patients, and *Pseudomonas* is significantly enriched in the oral cavity, gut, and urethra. Our research also suggested that microbes in different sites of the body are not independent entities and there may exist flora transfer between multiple sites and tumors in CC patients.

The vaginal microbiota related to bacterial vaginosis has emerged as a potential driver of persistent high-risk HPV infection and cervical neoplasia in women, characterized by decreased abundance of *Lactobacilli* and increased diversity of anerobic and facultative bacteria [20, 21]. Our results align with previous research, showing that cervical cancer patients lack *Lactobacillus* dominance in the vagina but have a diverse composition of other bacteria. *Lactobacillus* provides resistance to foreign bacteria and viruses (including high-risk HPV) by producing lactic acid and H_2O_2 to create a low pH environment and secreting large amounts of antimicrobial peptides [22, 23]. Depletion of *Lactobacillus* may lead to a pro-inflammatory environment that promotes the proliferation of CC and expression of HPV E6 and E7 oncogenes [24]. It has also been shown that *Lactobacillus* can integrate into the dense biofilm formed by pathogens and exert destructive and cytopathic effects, thereby reducing the occurrence and recurrence of genital tract inflammation [25]. According to the results of LEfSe, *Prevotella*, *Porphyromonas*, *Gardnerella*, *Proteus*, and *Anaerococcus* were identified as CC-specific vaginal biomarkers. The potential carcinogenic effects of these biomarkers have been reported by several studies. For example, *Prevotella* was significantly correlated with the expression levels of NF-κB and C-myc in cervical cells, influencing the occurrence of HPV infection and cervical lesions [26]. *Porphyromonas* played a causal role in pancreatic cancer development by protecting pancreatic ductal adenocarcinoma cells from reactive oxygen species-mediated cell death [27]. *Proteus* was associated with lymph node metastasis in HPV-positive oropharyngeal squamous cell carcinoma [28]. However, the specific roles of these biomarkers in the development and progression of CC are not yet fully understood and remain to be elucidated in the future.

Based on 16S rRNA sequencing analysis, significant changes in the composition of gut microbiota were observed in CC patients, with higher α-diversity than

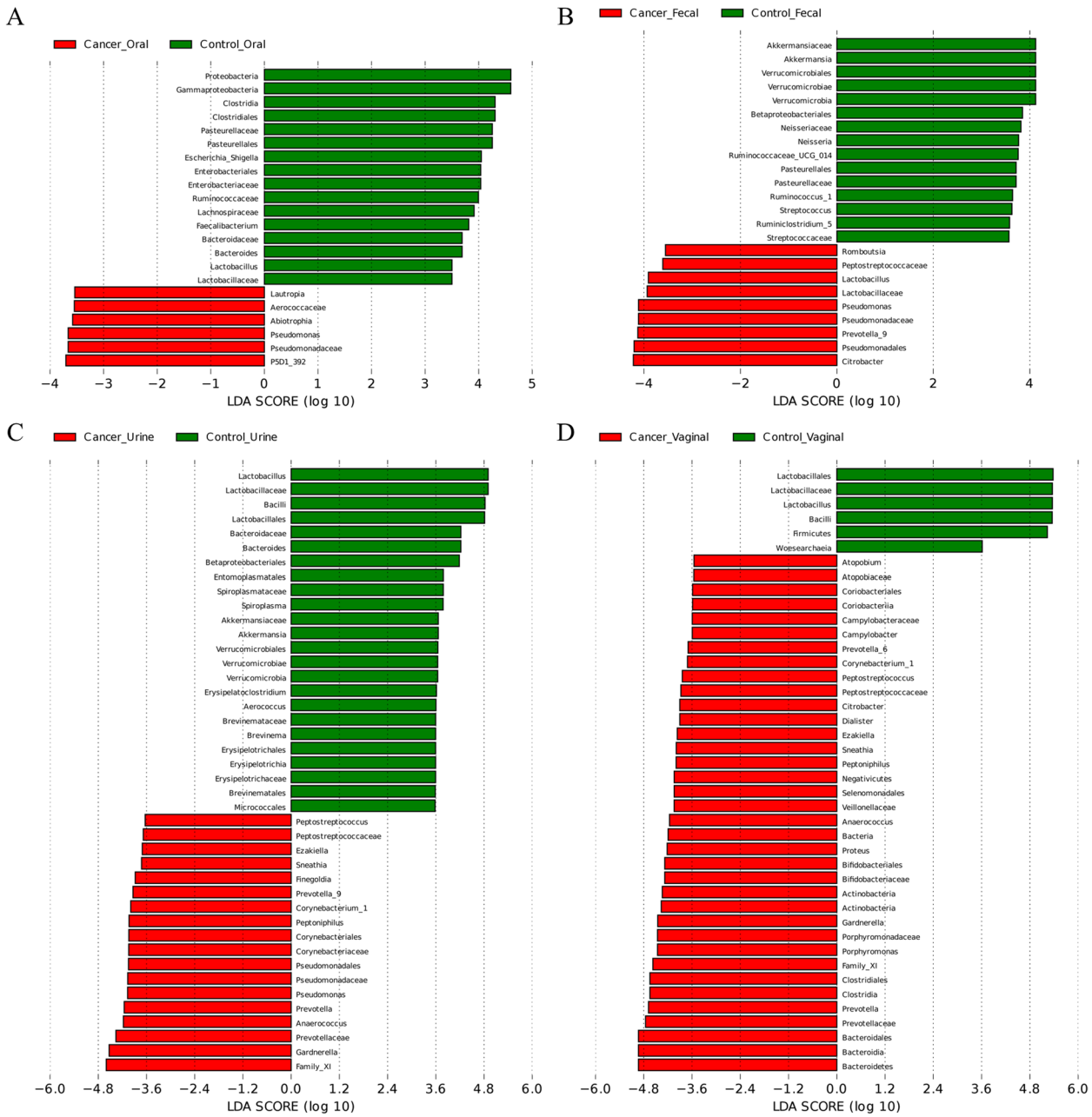

Fig. 4 LEfSe analysis identification of the microbial biomarkers of multi-site microbiomes in cervical cancer and healthy controls

controls, which was positively correlated with age [29, 30]. However, another study revealed a tendency for the microbiome α-diversity in CC patients to be lower than healthy controls [31]. We detected a lower α-diversity in the intestine of CC patients, and the abundance of *Citrobacter*, *Prevotella_9*, *Romboutsia*, *Lactobacillus*, and *Pseudomonas* were significantly higher in CC patients compared with healthy controls. The *Prevotella*-rich environment promotes T helper type 17 (Th17) immune responses and activates neutrophils [32], indicating that these microbiota might participate in the initiation or progression of CC. We suggested that *Prevotella* might be involved in the initiation and progression of CC by promoting chronic inflammation. *Lactobacillus* is generally considered to be a probiotic, but it also metabolizes dietary tryptophan, and the bacterial metabolites (indoles) produced have been reported to stimulate the aryl hydrocarbon receptor on tumor-associated macrophages, thus

inhibiting the release of interferon-γ by intra-tumoral $CD8^+$ T cells required to kill tumor cells and promoting the growth of pancreatic ductal carcinoma [33]. Although most studies suggest that gut microbiota is correlated with CC, the abundance of microbiota and the potential clinically relevant biomarkers identified were inconsistent. Considering diet is a pivotal determinant of the gut microbiota community [34], the difference in results may be partly due to different regional food cultures in these studies.

The association between dysbiosis of the oral microbiota and malignant tumors is the subject of extensive investigations [35]. Wei et al. [36] reported that the oral microbiota diversity of CC patients was significantly lower than that of controls, and several microbes, including *Fusobacterium*, *Campylobacter*, *Capnocytophaga*, *Veillonella*, *Streptococcus*, *Lachnoanaerobaculum*, *Propionibacterium*, *Prevotella*, *Lactobacillus*, and *Neisseria* were identified as CC biomarkers. Our results confirmed a lower α-diversity in the oral cavity of CC patients, but identified different CC biomarkers, including *Pseudomonas*, *Abiotrophia*, and *Lautropia.* As there were limited previous studies, the results on the relationship between oral microbiome and CC require replication.

Data from a study by Thomas-White et al. [37] suggested that the vaginal and UT microbiomes are interconnected. The UT and vaginal microbiotas not only share clonal pathogens but also share commensal organisms associated with vaginal health. Our results showed that with the occurrence of CC, the proportion of *Lactobacillus* in urine decreased, and the abundance of *Gardnerella*, *Anaerococcus*, and *Prevotella* increased, consistent with changes in vaginal flora. The current research on the urinary microbiome is focused mainly on the occurrence and pathogenesis of UT inflammation and UT malignant tumors [18, 38]. Possible carcinogenic mechanisms include dysbiosis of the urethral microbiota, which disrupts the physiological immune barrier formed by commensal bacteria and the immune system, leading to sustained inflammatory responses and DNA damage [39]. The relationship between α-diversity of urine microbiome and malignancies was inconsistent. Some observed increased bacterial richness in cancer patients than controls [40]. Others found lower species richness in cancer patients [41]. In this study, α-diversity showed a trend to decrease with disease progression (Supplementary Fig. 1D, F), however, there was no difference in microbial α-diversity between CC patients and controls (Fig. 3C). It may be that the abundance of specific bacteria in urine is more important than the total number of bacteria taxa present. Mechanisms related to the effect of UT microbiota on CC development and progression need to be determined in future studies.

Early studies showed that bacteria are present in tumor tissue, with most present in cancer cells and immune cells [42]. It is suggested that the intratumor microbiome may originate from multiple mucosal organs, with three main potential sources of intratumoral microbiota. First, tumorigenesis and a variety of other factors cause damage to the mucosal barrier, which may provide a pathway for opportunistic organisms to colonize the tumor, creating an intra-tumoral microbiota [7]. Alternatively, the tumor tissue has a rich blood supply, which provides conditions for microorganisms to enter the tumor tissue through the circulatory system [43]. The third possible source of the microbiome in the tumor arises from the high degree of similarity of the bacterial profile in the tumor and that of the normal adjacent tissues (NATs) [42]. Bacteria in cervical cancer tissues were reported mainly arise from vaginal mucosa for the breach of mucosal barriers may provide access [44]. Multiple sites can serve as potential reservoirs of vaginal microorganisms through the oral-vagina, gut-vagina, and bladder-vagina axis [14, 36].By comparing the microbiome between matched samples, we found that a portion of intratumor bacteria correlated with microorganisms in multiple mucosal organs (Supplementary Table 4), suggesting bacteria may migrate from these mucosal organs into tumors (Fig. 5). However, some intratumor bacteria such as *Ralstonia* and *Sediminibacterium*, are rare in mucosal organs, meaning there may be other sources of intratumor microbes. However, owing to technical limitations, it was difficult to achieve complete intratumor bacteria genome by metagenomic sequencing because of its low biomass compared to host information. We will make traceability analysis between representative bacteria cultivated from tumor tissues and strains from suspected sites using single-nucleotide variations to further investigate the source of intratumor bacteria in the future.

The differences in microbial composition and biodiversity of various mucosal organs between CC patients and controls may have broad implications for the search for biomarkers for early screening of CC. However, this study has some limitations. First, the CC patients and controls exhibited different characteristics in terms of age and HPV status. The mean age of controls (45.64 ± 3.50 years) was younger than that of CC patients (53.03 ± 13.09 years) (Supplementary Table 1). It is generally believed that the age influence on the microbiota could be related to hormones. The microbiome differences between CC and controls could partly be due to age. CC is the most common HPV-related malignancy, while there were no HPV-positive controls (Supplementary Table 1). A group including women infected with HPV without cervical dysplasia could be better to identify particular bacteria taxa

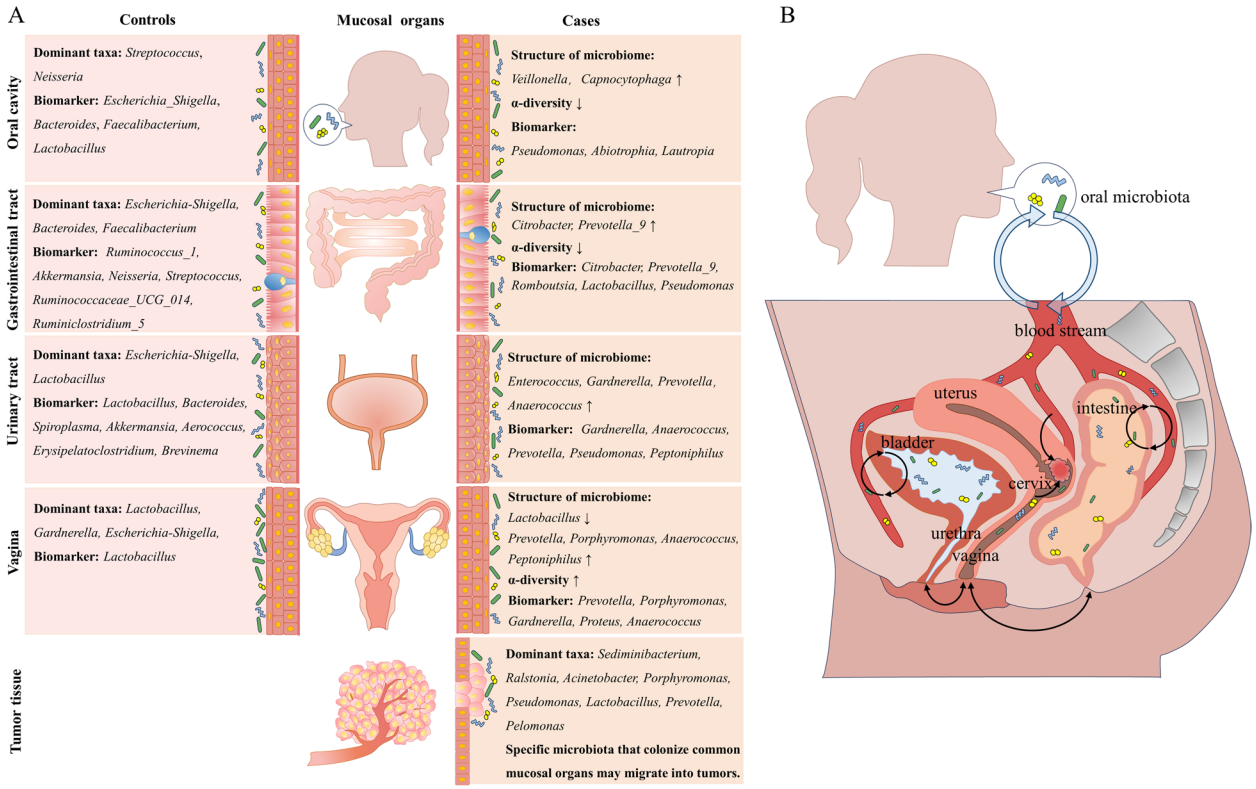

Fig. 5 Dysbiosis of microflora in multiple mucosal organs and multi-site microbiota metastasis in cervical cancer patients

associated with CC. Second, details of many factors such as sexual orientation, sexual activity, number of sexual partners, age at first term pregnancy, diet and/or nutrition were not available. There was no specific analysis of the association between risk factors and bacteria in multi-organs. Third, due to the case-control design of this study, it is also difficult to determine the time sequence between the dysbacteriosis of various organs and the occurrence of CC.

Future research should explore the role of microbiota biomarkers of multiple mucosal organs in tumorigenesis, in order to facilitate the development of rational targets for prevention, screening and diagnosis. With the application of fecal and vaginal microbiota transplantation techniques, probiotic therapy intervention is also an interesting research field in the future.

Conclusions

In conclusion, we revealed that CC patients have unique microbiome in multiple mucosal organs. The bacterial diversity of urine in CC patients was found to be related to multiple clinical features. Further studies are warranted to validate the observed difference in microbiota and elucidate their biological significance and potential mechanisms in CC.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-12915-1.

Supplementary Material 1.

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Authors' contributions

LZ designed the experiment and supervised the study. ZYD collected biological samples from subjects, CHA, KML, MPJ, XRW, CFZ, ZL provided the clinical information, PL analyzed the sequencing results and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset generated and analyzed in this study are temporarily unavailable to the public, as they are also being used for an ongoing research project, but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study adhered to the principles outlined in the Declaration of Helsinki and was reviewed and approved by the Research Ethics Committee of the Third Affiliated Hospital of Kunming Medical University (Approval no. KYSC202161). Written informed consent was obtained from all participants included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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