Gut Pathogens

RESEARCH Open Access

Long-read 16S rRNA amplicon sequencing reveals microbial characteristics in patients with colorectal adenomas and carcinoma lesions in Egypt

Asmaa A. El Leithy[1*], Amira Salah El-Din Youssef[2*], Auhood Nassar[2], Ramy K. Aziz[3,4], Nadin M. Khaled[1], Mina T. Mahrous[1], Ghobrial N. Farahat[1], Aya H. Mohamed[1] and Yasser Mabrouk Bakr[5]

Abstract

Background Colorectal cancer (CRC) is among the five leading causes of cancer incidence and mortality. During the past decade, the role of the gut microbiota and its dysbiosis in colorectal tumorigenesis has been emphasized. Metagenomics and amplicon-based microbiome profiling provided insights into the potential role of microbial dysbiosis in the development of CRC.

Aim To address the scarcity of information on differential microbiome composition of tumor tissue in comparison to adenomas and the lack of such data from Egyptian patients with CRC.

Materials and methods Long-read nanopore sequencing of 16S rRNA amplicons was used to profile the colonic microbiota from fresh colonoscopic biopsy samples of Egyptian patients with CRC and patients with colonic polyps.

Results Species richness of CRC lesions was significantly higher than that in colonic polyps (p-value = 0.0078), while evenness of the CRC group was significantly lower than the colonic polyps group (p-value = 0.0055). Both species richness and Shannon diversity index of the late onset CRC samples were significantly higher than those of the early onset ones. The Firmicutes-to-Bacteroidetes (F/B) ratio was significantly higher in the CRC group than in the colonic polyps group (p-value = 0.0054), and significantly higher in samples from early-onset CRC. The *Enterococcus* spp. were significantly overabundant in patients with rectal cancer and early-onset CRC, while *Staphylococcus* spp. were significantly higher in patients with sigmoid cancer and late-onset CRC. In addition, the relative abundance of *Fusobacterium nucleatum* was significantly higher in CRC patients.

Conclusion Differentiating trends were identified at phylum, genus, and species levels, despite the inter-individual differences. In summary, this study addressed the microbial dysbiosis associated with CRC and colonic polyps groups, paving the way for a better understanding of the pathogenesis of early and late-onset CRC in Egyptian patients.

*Correspondence:
Asmaa A. El Leithy
asmaa.elleithy@must.edu.eg
Amira Salah El-Din Youssef
amira.salah@nci.cu.edu.eg

Full list of author information is available at the end of the article

Keywords Microbiome, 16S rRNA, Colorectal cancer, Colonic polyps

Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer, in terms of incidence, and the second leading cause of cancer mortality (9.3% of the total cancer deaths). In men, it is the third cancer site with the highest age-standardized rate in countries with higher human development index [1].

CRC mostly arises from colorectal polyps (adenoma) as the polyps are prone to malignant carcinoma transformation [2]. Genetic mutations associated with the disease progression in Egyptian patients with CRC have been recently described [3]. CRC development is multifactorial, with a strong genetic component. However, it is also one of the most lifestyle-affected cancers, since the colon is directly connected to diet and various dietary pollutants. Additionally, the past decade emphasized the role of the gut microbiota and its dysbiosis in colorectal tumorigenesis, which might be a causative change [4].

Recent advancements in high-throughput sequencing technologies, such as shotgun metagenomics and 16S rRNA amplicon sequencing, have notably improved the understanding of the role of microbiome in CRC progression [5]. Fortunately, in the past few years, long-read sequencing, such as the nanopore technology, has improved in accuracy and dropped in price. It offers many advantages over the most widely used short-read sequencing approaches, most importantly the ability to resolve differences between species with near-identical rRNA variable regions, since nanopores allow the sequencing of the full 16S rRNA gene, eliminating phylogenetic biases [6].

Metagenomics and amplicon-based microbiome profiling provided insights into the potential role of microbial dysbiosis in the development of CRC. Dozens of studies delineated specific bacterial taxa and CRC-associated functional pathways [7]. In addition, biomarkers for early detection and prevention of CRC are also being identified. For example, a panel of 16 bacterial markers could differentiate between CRC patients and healthy controls with 92% accuracy [8].

Bacterial phyla, such as Firmicutes and Bacteroidetes, were pinpointed as underrepresented in patients with cancer, compared with healthy individuals. However, *Prevotella copri, Mansonia uniformis, Fusobacterium nucleatum*, and specific strains of *Escherichia coli* have been described as overabundant in cancer groups [9].

In addition to the altered microbiota makeup, pathogenic bacterial species might contribute to the emergence of CRC such as several *Bacteroides* species (*B. vulgatus,B. fragilis*, and *B. stercoris*), *Bifidobacterium angulatum*, some *Ruminococcus* species, *Fusobacterium prausnitzii* [10]. Such microbes are believed to induce CRC tumorigenesis by promoting the proliferation of the epithelial cells, producing epithelial barrier damage and causing inflammation. Moreover, different toxins may damage DNA, stimulating the pro-tumorigenic effect. For example, *Bacteroides fragilis* toxin is reported to activate Wnt and NF-kB signaling pathways and induce the epithelial release of pro-inflammatory molecules [11].

A growing body of evidence supports that the microbiome can influence response to immunotherapy and chemotherapy [12, 13]. Modulating the microbiome may provide methods to increase the efficacy of treatments, reduce treatment toxicities, and even prevent carcinogenesis. While research on the fecal microbiome has been frequently conducted, little is known about the role of tissue microbiota in determining disease associations and the diagnostic and therapeutic potential of the microbiome in Egyptian patients with CRC [14].

In Egypt, only a handful studies have addressed the microbiome involvement in CRC, yet these studies were based on fecal microbiome profiling, or fecal analysis by real-time PCR [15–19] but none profiled the tumor tissue.

Thus, this study was launched to address the scarcity of information about the tissue microbiome composition by using long-read sequencing to compare the microbiomes of CRC tumors and polyps, specifically tackle the lack of any such data from Egyptian patients with CRC, given the importance of geographical and dietary factors shaping the microbiome. In addition, we identified microbiome variations associated with early and late-onset CRC, as well as anatomical tumor site.

Materials and methods

Ethics statement

All protocols and procedures were approved by the Institutional Review Board (IRB approval number: CB2309-302-071) of National Cancer Institute (NCI), Cairo University, Egypt. Written informed consent was obtained from each participant before their enrolment in this study.

Sample collection and description

Fresh colonoscopic biopsy samples (n = 15) from CRC patients and patients with colonic polyps (n = 14) were recruited from the NCI of Egypt. The collected biopsies were stored in MACS Tissue Storage Solution in a − 80 freezer until DNA extraction. All the participants' clinicopathological data were collected from their National Cancer Institute (NCI) clinical records.

DNA extraction

DNA was isolated from the collected biopsies using the QIAamp® DNA mini kit (Cat. No. 51304, Qiagen, Germany) following the manufacturer's instructions. The concentration of the purified DNA was measured using Qubit® 3.0 Fluorometer (Cat. No, Q33216, Thermo Fischer Scientific Inc., USA) with Qubit™ dsDNA BR assay kit (Cat. No. Q32850, Thermo Fischer Scientific Inc., USA).

Amplification of 16S rRNA

PCR amplification for the 16S rRNA gene was performed using the 27 F/1492R primer set from the 16S Barcoding Kit (SQK-RAB204; Nanopore Technologies, Oxford, UK) and PCRBIO HS Taq Mix Red (PCR Biosystems Ltd., London, Oxford, UK) according to the manufacturer's protocol in a reaction volume of 50 µL consisting of 10 ng of genomic DNA and 1 µl 16S barcoded primers at 10 µM. The thermal profile for the amplification was as follows: initial denaturation at 95 °C for 2 min, 30 cycles of 95 °C for 20 s, 55 °C for 30 s, and 72 °C for 45 s, followed by a final extension at 72 °C for 5 min, using MyCycler Thermal Cycler (Bio-Rad, California, USA) following the manufacturer's instructions.

Table 1 Demographic and clinical data of the studied patients

	CRC ***n*** **= 15**	**Colonic polyps** ***n*** **= 14**
Age: Early age ≤ 45 Late age > 45	4 (27%) 11 (73%)	3 (21%) 11 (79%)
Sex Male Female	5 (33%) 10 (67%)	6 (43%) 8 (47%)
Histological type	Adenocarcinoma 12 (80%) Mucinous adenocarcinoma 1 (7%) Signet Ring adenocarcinoma 2 (13%)	Typical lesion 5 (34%) Atypical lesion 6 (45%) Mixed lesions 3 (21%)
Site Colon Rectum Sigmoid	7 (47%) 6 (40%) 2 (13%)	13 (93%) 0 (0%) 1 (7%)
Grade II NA	13 (87%) 2 (13%)	-
Metastasis Yes No	0 (0%) 15 (100%)	-
Recurrence Yes No	5 (33%) 10 (67%)	-

Library preparation and sequencing for the 16S rRNA amplified PCR products

The PCR amplicons were pooled then purified using AMPure XP (Beckman Coulter, Indianapolis, IN, USA) and quantified using Qubit 4 (Thermo Fischer Scientific). A total of 200 ng DNA was used for library preparation; the resulting library was sequenced with MinIon™ sequencer (Nanopore Technologies, Oxford, UK) for almost 24 h using R9.4.1 flow cells (FLO-MIN106; Oxford Nanopore Technologies) according to the manufacturer's instructions. Minknow software version 22.12.7 (Oxford Nanopore Technologies) was used for real-time base-calling for sequenced data.

Bioinformatics and statistical analysis

The Guppy software V6.4.6 (Oxford Nanopore Technologies) was used for base-calling, adapter/barcode trimming, and then to generate FASTQ-formatted sequence files, with an accurate base-calling model and read filtering of min_ score = 9 and reads below Q9 were eliminated. The FASTQ reads were assigned to their taxonomic group by alignment to the NCBI 16S database.

After relative taxon abundance data were obtained, all data were analyzed for statistical significance and visualized by publicly available packages in the R environment (https://www.r-project.org/) and the RStudio software (version 2022.02.0 Build 443). The following R packages were used: *readxl, dplyr, tidyverse, ggplot2, ggpubr, corrplot, magrittr, pheatmap*, and *coin*.

All comparisons between two variables were tested for statistical significance by the non-parametric Mann-Whitney test, while comparisons between multiple variables were tested for significance by the Kruskal-Wallis test, followed by *post hoc* Mann-Whitney tests with Tucky adjustment. Correlation analysis was performed by the Spearman non-parametric method.

Results

Patients' clinical data and metadata

Age, sex, histological type, site, grade, and state of metastasis or recurrence for all participants are summarized in Table 1.

Microbial composition of the studied patients with CRC and colonic polyps

Nanopore sequences of the full 16S rRNA gene from all the study participants were generated and filtered for quality, then assigned to different taxonomic levels. Despite obvious inter-individual differences, differentiating trends could still be identified. At the phylum level, Firmicutes and Actinomycetota were overabundant in the CRC group. At the family level, several samples from the CRC group were highly enriched in family *Enterococcaceae*, while *Bacteroidaceae* was more represented

in the colonic polyps (Fig. 1). The significantly abundant bacterial phyla and families in CRC and colonic polyps are presented in Supplementary Table 2.

At the genus level, multiple genera were significantly associated with CRC tissue, such as *Enterococcus,Cutibacterium,Staphylococcus,Corynebacterium,Peptostreptococcus*, and *Fusobacterium*. On the other hand, genera associated with colonic polyps included *Proteus,Prevotella,Bacteroides*, and *Macrococcus* (Fig. 2). Abundant bacterial genera and species among CRC and colonic polyps are presented in Supplementary Table 3.

Signature microbiome profiles in patients with CRC and colonic polyps

Using correlation analysis of taxon abundance profiles within different samples delineated five clusters, three

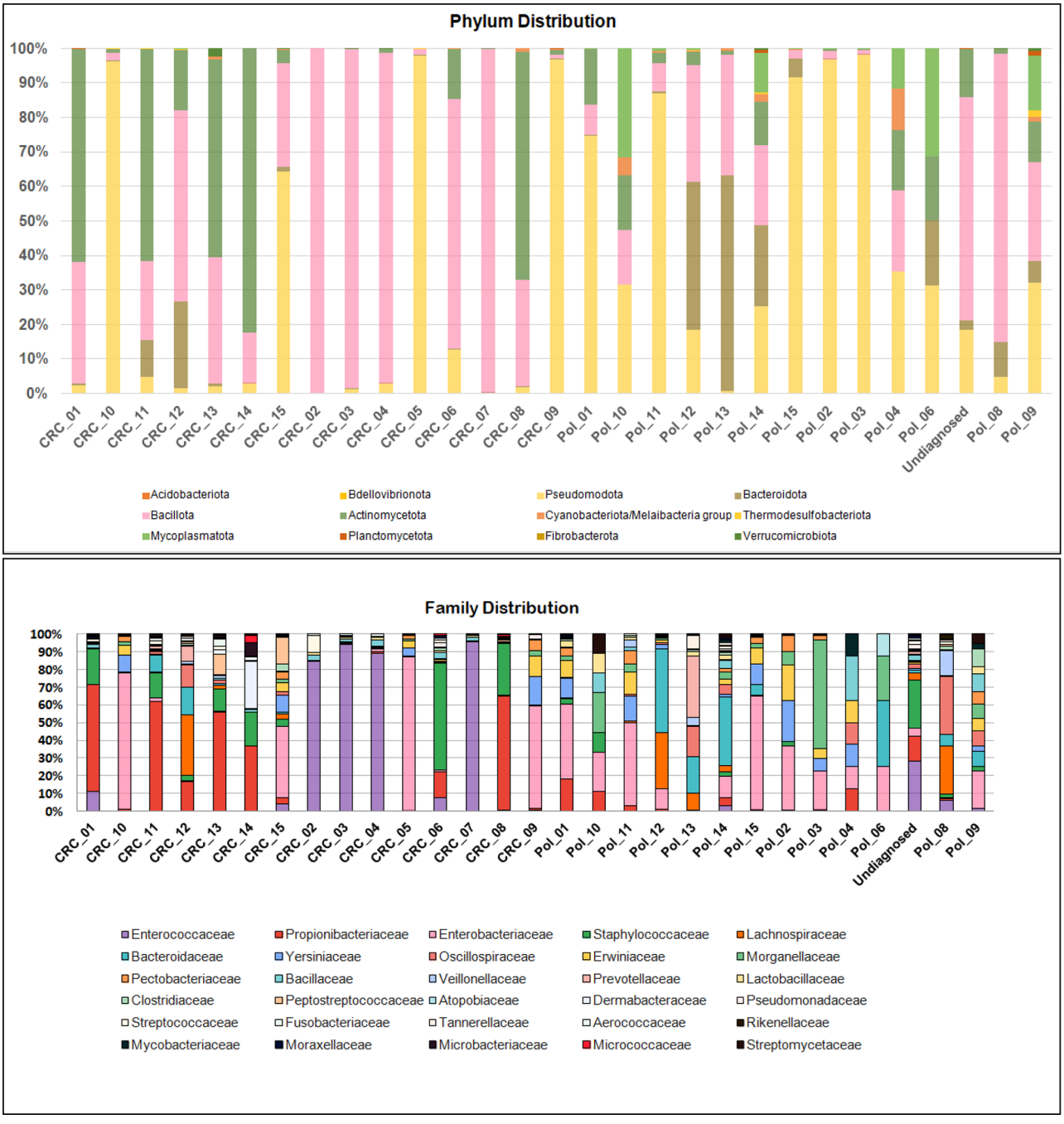

Fig. 1 Distribution of bacterial phyla and families in CRC and colonic polyps groups. Y axis: relative abundance (% of total reads) of reads assigned to the indicated phyla, after unassigned sequences were excluded. X axis: different samples from patients with CRC, polyps, and one ambiguous, undiagnosed case

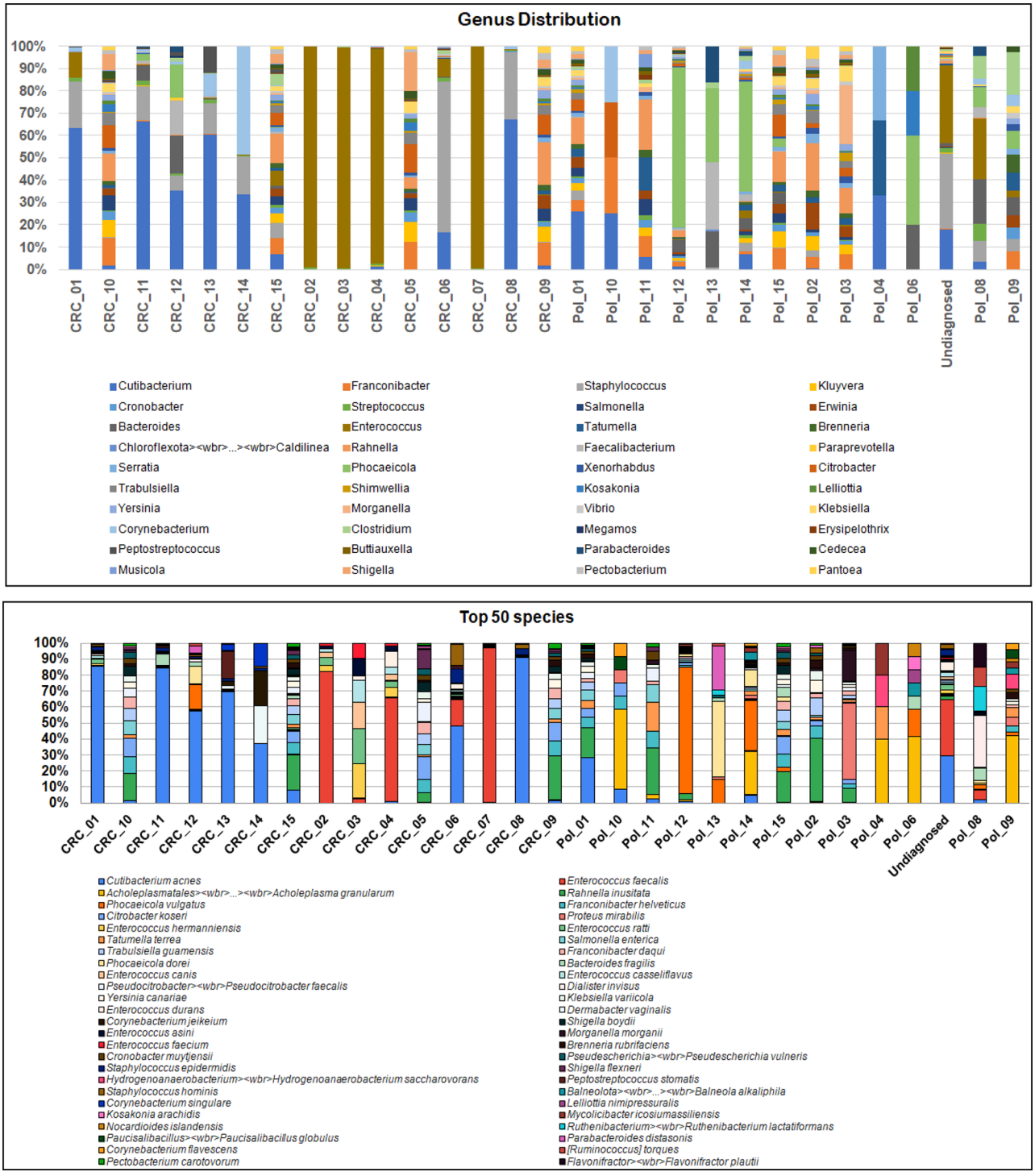

Fig. 2 Genus distribution (Top panel) and the top 50 species (bottom panel) of the current studied samples

of which are clearly associated with the type of lesion (Clusters B, D, and E in Fig. 3). Similar clustering analysis (Supplementary Fig. 1) identified two clear clusters in tissues of the CRC groups. In Cluster 1, *Cutibacterium* (17.01%), *Staphylococcus* (8.60%), *Corynebacterium* (2.86%), *Dermabacter* (1.41%), and *Peptostreptococcus* (0.91%) significantly dominated the tissue microbiota of CRC patients (samples CRC_08, CRC_11, CRC_12, CRC_13, CRC_14, four of which are members of Correlation Cluster D in Fig. 3). Cluster 2 was characterized by a significant overabundance of *Enterococcus* (24.28%) in the tissue of CRC patients (samples CRC_02, CRC_03,

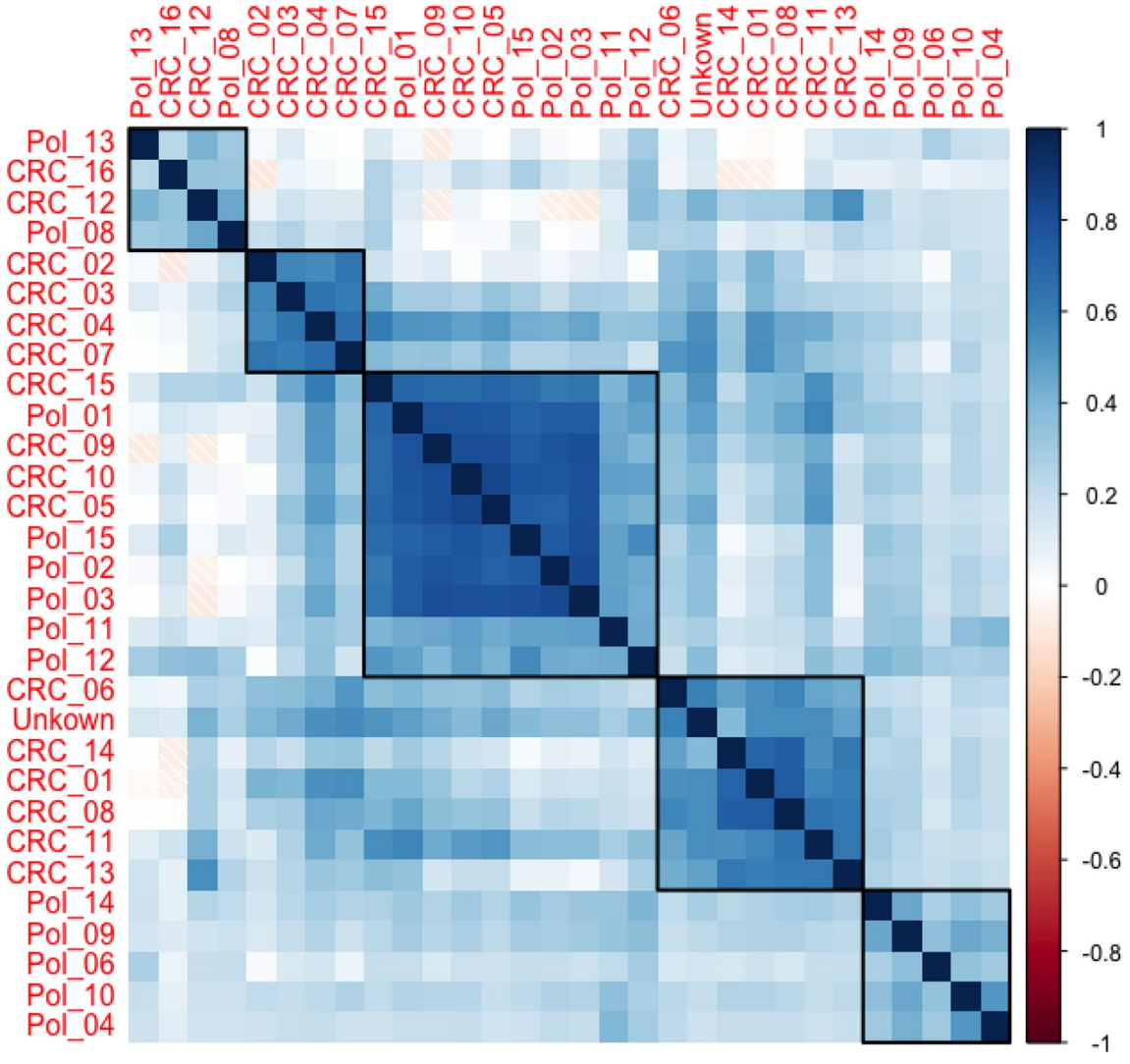

Fig. 3 A color-coded correlation plot indicates five clusters of correlated relative abundance within the microbiome profiles of samples from CRC tissue and colonic polyps

CRC_04, CRC_07, which are all members of Correlation Cluster B in Fig. 3).

Alpha diversity variations among samples

Alpha diversity (at the species level) was analyzed and compared between different samples. Overall, species richness within the CRC group was significantly higher than that of the colonic polyps group (Mann Whitney p-value = 0.0078, Fig. 4A), while evenness of CRC group was significantly lower than that of the colonic polyps group (Mann Whitney p-value = 0.0055, Fig. 4B). Moreover, both species richness and Shannon diversity index of the late onset CRC samples were significantly higher than that of early onset ones (Mann-Whitney p-values = 0.02 and 0.013 respectively, Fig. 4C-D).

Firmicutes/Bacteroidetes (F/B) ratio

The Firmicutes-to-Bacteroidetes (F/B) ratio (or Bacillota-to-Bacteroidota, by the current nomenclature) was among the earlier microbiome biomarkers to be considered as it correlates with several health conditions. In this study, the F/B ratio was significantly higher in the CRC group than in the colonic polyps group (p-value = 0.0054). The F/B ratio was also significantly higher in early-onset CRC patients than in late-onset CRC patients (Fig. 5).

Anatomical site and the age of diagnosis vs. microbial taxon composition

We also investigated the association between the relative taxon abundance and the anatomical site. The relative abundance of *Staphylococcus* spp. and *Cutibacterium acnes* were significantly higher in samples from sigmoid cancer, while *Enterococcus cecorum* and *Enterococcus*

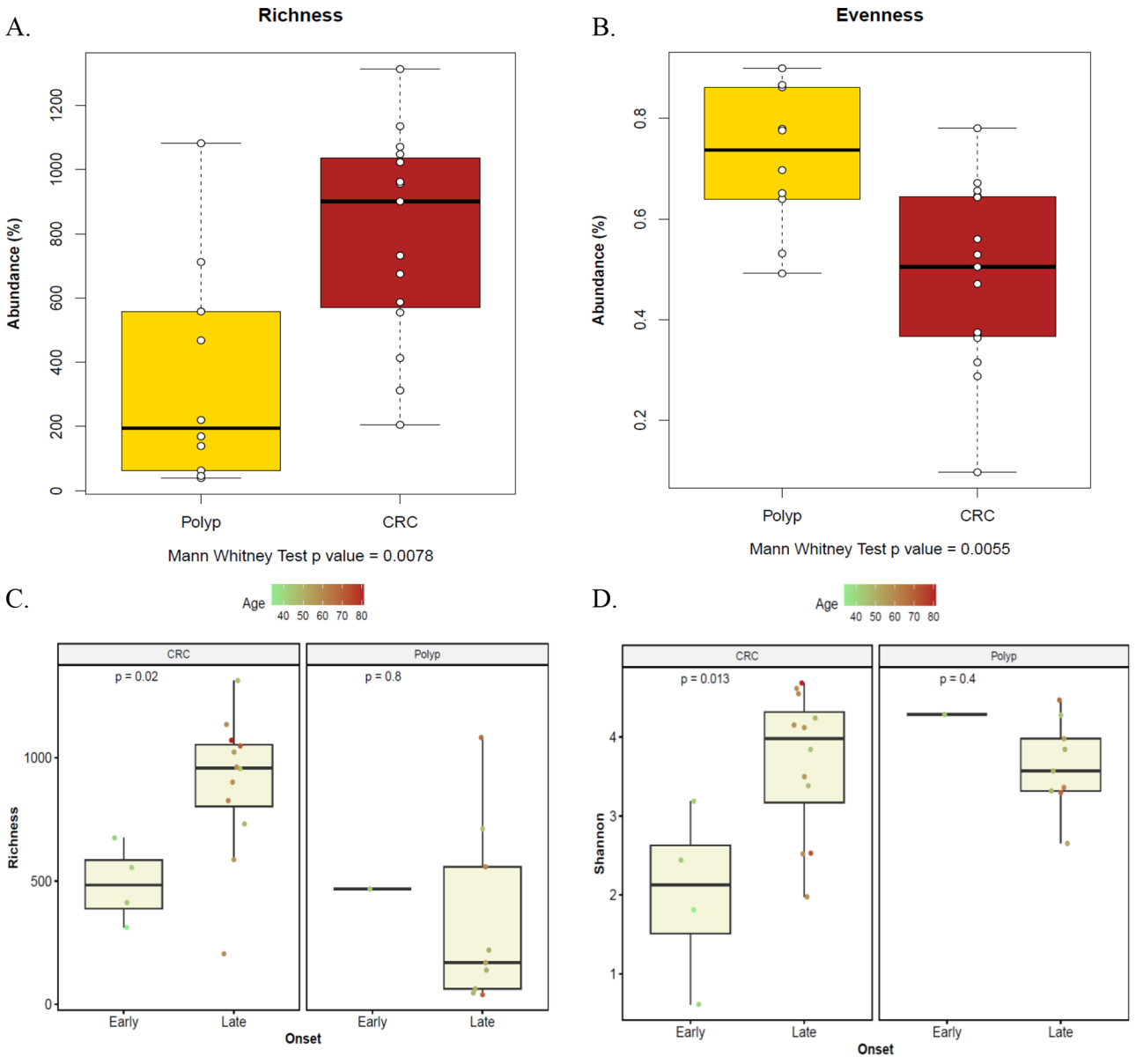

Fig. 4 Alpha diversity analysis: species richness index (**A** and **C**), Shannon evenness index (**B**), and Shannon diversity index (**D**) in CRC and colonic polyp groups. Differencess between early and late-onset tumors are specifically shown in panels **C** and **D**. All differences were tested for significance by the non-parametric Mann-Whitney test. *p*-values are shown

columbae were significantly overabundant in samples with rectum-cancer. The relative abundance of *Fusobacterium nucleatum* was significantly higher in samples from colon cancer (Fig. 6).

Finally, we investigated the possible differential abundance of some microbial taxa between early (≤45 years) and late (>45 years) onset of CRC and colonic polyps. At the genus level, *Staphylococcus,Peptostreptococcus*, and *Brevibacterium* were significantly more abundant in samples from patients with late onset of CRC, while *Enterococcus* and *Lactobacillus* were significantly more abundant in samples from patients with early onset of CRC (Fig. 7A). At the species level, *Cutibacterium acnes* and *Staphylococcus hominis* were significantly more abundant in samples from patients with late onset of CRC, while *Enterococcus cecorum, Enterococcus columbae, Enterococcus faecalis*, and *Enterococcus faecium* were significantly associated with early onset of CRC (Fig. 7B).

Discussion

CRC is linked to changes in microbial composition, often known as dysbiosis [20, 21]. Different lifestyle-related factors, such as diet and body weight, may alter the gut microbiota and influence the risk of developing CRC [22]. Genetic and epigenetic alterations brought on by

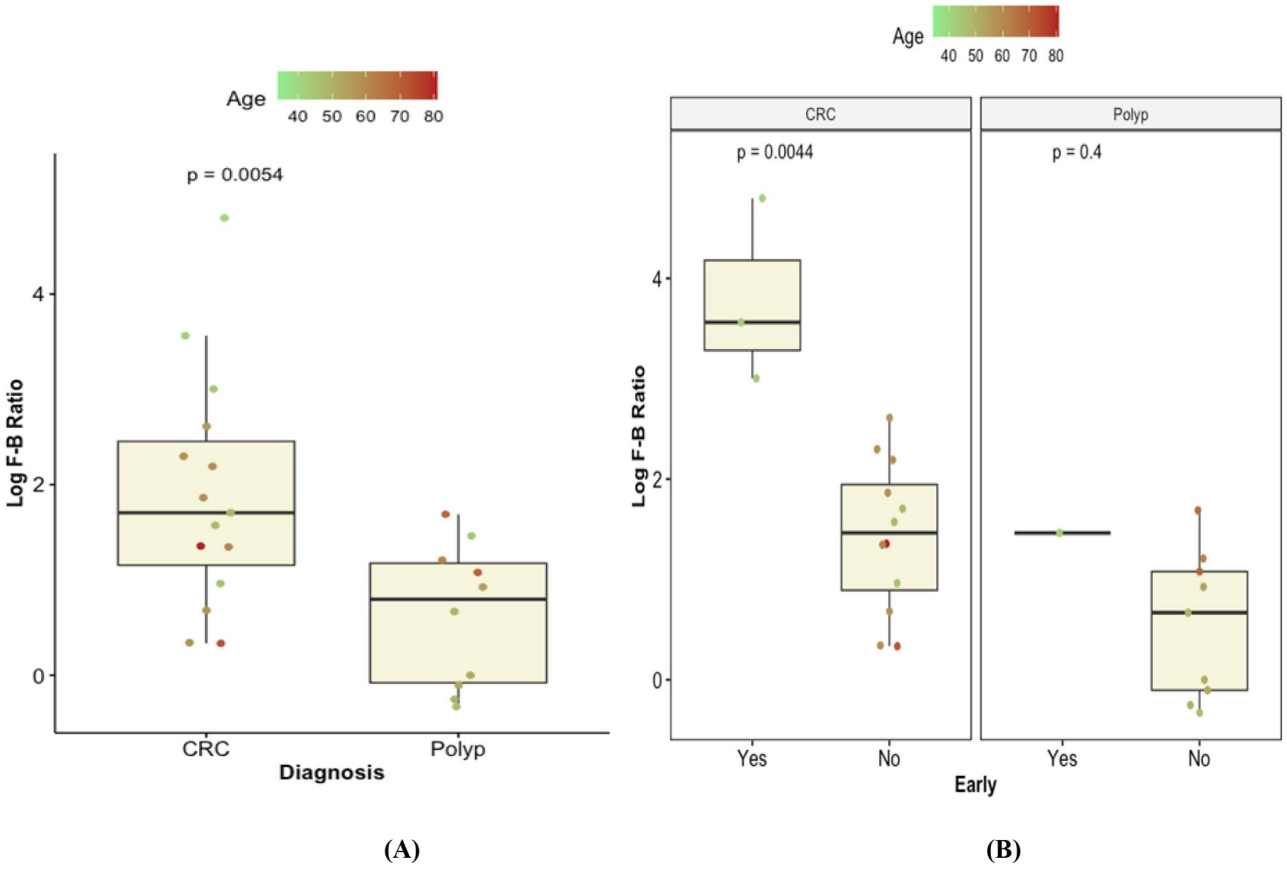

Fig. 5 Firmicutes/Bacteroidetes(F/B) ratio (**A**) between CRC and colonic polyps groups, and (**B**) between the early and late onset of CRC and colonic polyps. *p*-values were calculated by Mann-Whitney test. (Yes) refers to early onset, and (No) refers to late onset, while the patient age is used as color gradient for each sample point

genotoxic stress to the gut microbiota or metabolites in the intestinal environment may result in cancer [23], and the development of CRC may be influenced by the overabundance of particular strains [24]. Most of the findings and associations about the microbiome involvement in CRC, however, are based on studies on fecal samples, which may represent the microbial diversity in the colon, but do dilute the actual composition at the cancerous or adenomatous tissue.

Although a number of excellent studies have identified polyp *vs.* CRC tissue microbiotas, the vast majority—to the best of our knowledge—relied on short-read sequencing technologies. Thus, it offers a broad picture of microbial composition, but—whether it relies on V3-V4 hypervariable region or other variable regions of the 16S rRNA gene lacks sufficient sequence length to resolve many bacterial species. We believe that our approach of using full-length 16S rRNA gene sequencing strengthens some of the prior findings by providing a long-read-based analysis, and adds higher taxonomic resolution at the species level. For example, Hua et al. used Illumina sequencing of the V3–V4 variable region of the 16S rRNA gene to characterize the microbiota differences along the adenoma-carcinoma sequence [25]. In addition, Zhong et al. performed 16S rRNA gene sequencing in normal colorectal mucosa and tissue of colorectal polyps as well as in feces. Their work revealed that *Fusobacterium* and *Streptococcus* were lower in feces both in patients with colorectal polyp and healthy individuals, when compared to those in the normal mucosa in the two groups or in polyp tissues. However, their study did not include CRC tissue samples [26].

Long-read sequencing has just started to be implemented in profiling the microbiota of different body sites or tissues. A recent study conducted Illumina MiSeq sequencing of the V4 region of the 16S rRNA gene to analyze mucosal biopsies collected from multiple colon sites, including healthy controls. This study reported significant alpha diversity differences between CRC and controls but found no clear separation between CRC and polyps. Further characterization of the *Fusobacterium* species and subspecies was performed by MinION nanopore sequencing, confirming their enrichment in CRC, which also agrees with our findings [27]. Another study by Wei et al. used long-read sequencing to classify the fecal microbiota changes associated with colonic

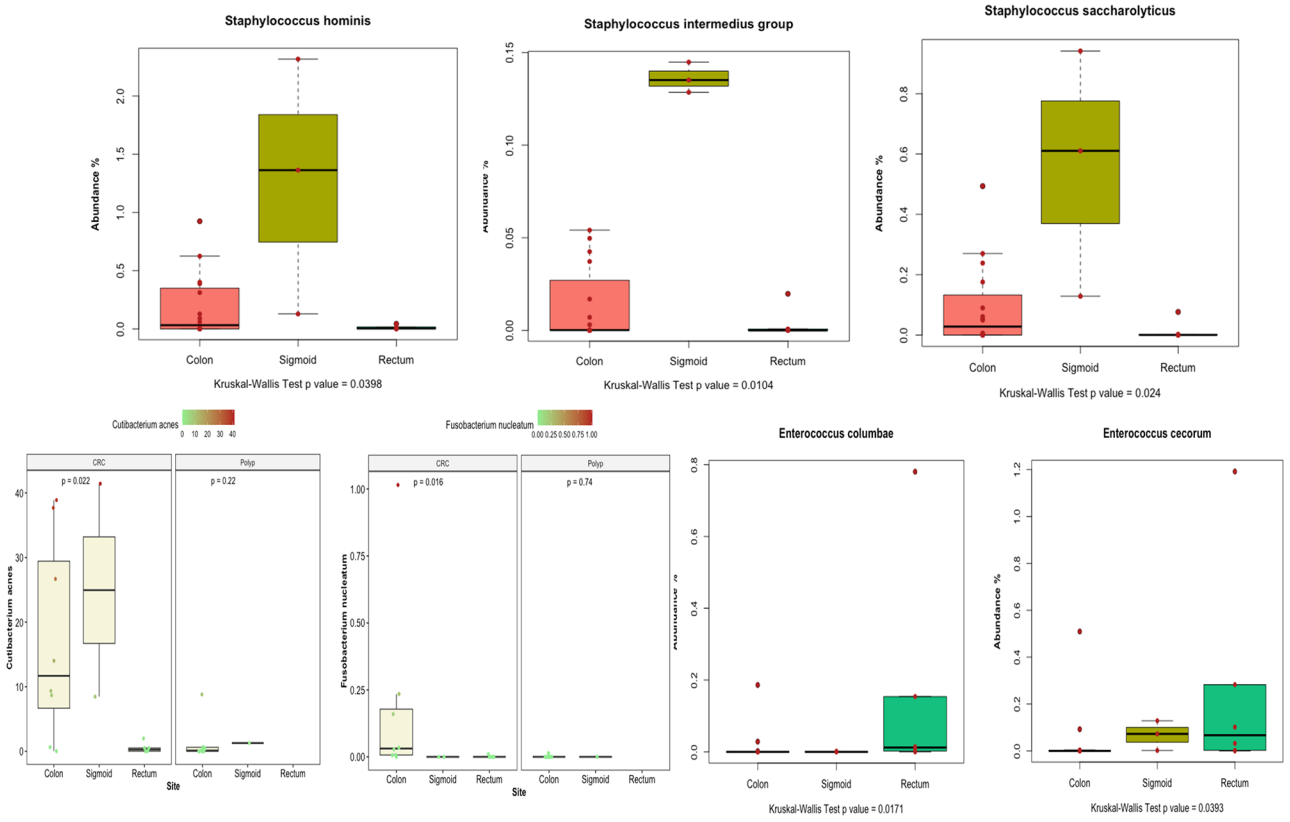

Fig. 6 Taxon abundance in CRC and colonic polyps groups split by anatomical site

adenomatous polyps. Their work provided a broader comparison, including healthy controls, occult blood patients, and adenomatous polyp cases. However, their study did not include CRC tissue samples [28].

The current study used long-read amplicon sequencing to identify bacterial clades at multiple taxon levels (up to the species level) from the tissue microbiome of CRC and colonic polyps, with some initial insights on early- vs. late-onset disease. Our results showed a significant variation in bacterial abundance between the two groups and the age subgroups. The major bacterial phyla detected in this study were Firmicutes, Pseudomonadota, Actinomycetota, Bacteroidetes, and Mycoplasmatota in both the colonic polyps and CRC group, consistent with Russo et al. [29].

It is to be noted that shotgun metagenomics methodologies are often superior to 16S amplicon-based ones as they offer insight into the differential abundance of genes, pathways, and subsystems. However, shotgun metagenomic sequencing is more suitable to stool samples than to tissue samples, as DNA extracted the latter will mostly represent the human host/tumor DNA rather than microbial DNA. Thus, we believe that the choice of long-read nanopore sequencing was the most appropriate for our goal of accurately identifying taxonomic differences between cancerous and adenomatous tissues.

We found that the relative abundance of Firmicutes and Actinomycetota was significantly higher in the CRC group compared with the colonic polyps group. On the other hand, the relative abundance of Pseudomonadota, Bacteroidetes, and Mycoplasmatota was substantially higher in the colonic polyps than in the CRC lesions. A published study reported the abundance of Bacteroidetes in colon cancer, contrary to our findings about its higher relative abundance in patients with colonic polyps; however, that study—like many others—relied on stool analysis and not tumor tissue [30]. As mentioned above, stool samples, while used as proxy for the gut microbiome, are not optimal in cases of localized tumors, as the same intestine will have distinct microbiome signatures at different sites, as confirmed in this work (Fig. 6).

Our data showed that the Firmicutes-to-Bacteroidetes (F/B) ratio was significantly higher in the CRC lesions than in the colonic polyps; this finding is in agreement with previous reports on the higher abundance of bacteria belonging to the Firmicutes phylum in CRC tumors [31]. In line with our findings, Quaglio et al. reported that patients with CRC have shown enrichment with Firmicutes and Bacteroidetes [32]. Of note, a study on Egyptian patients identified a significant reduction in "beneficial Firmicutes" in ulcerative colitis, colorectal adenoma, and CRC when compared to controls [19].

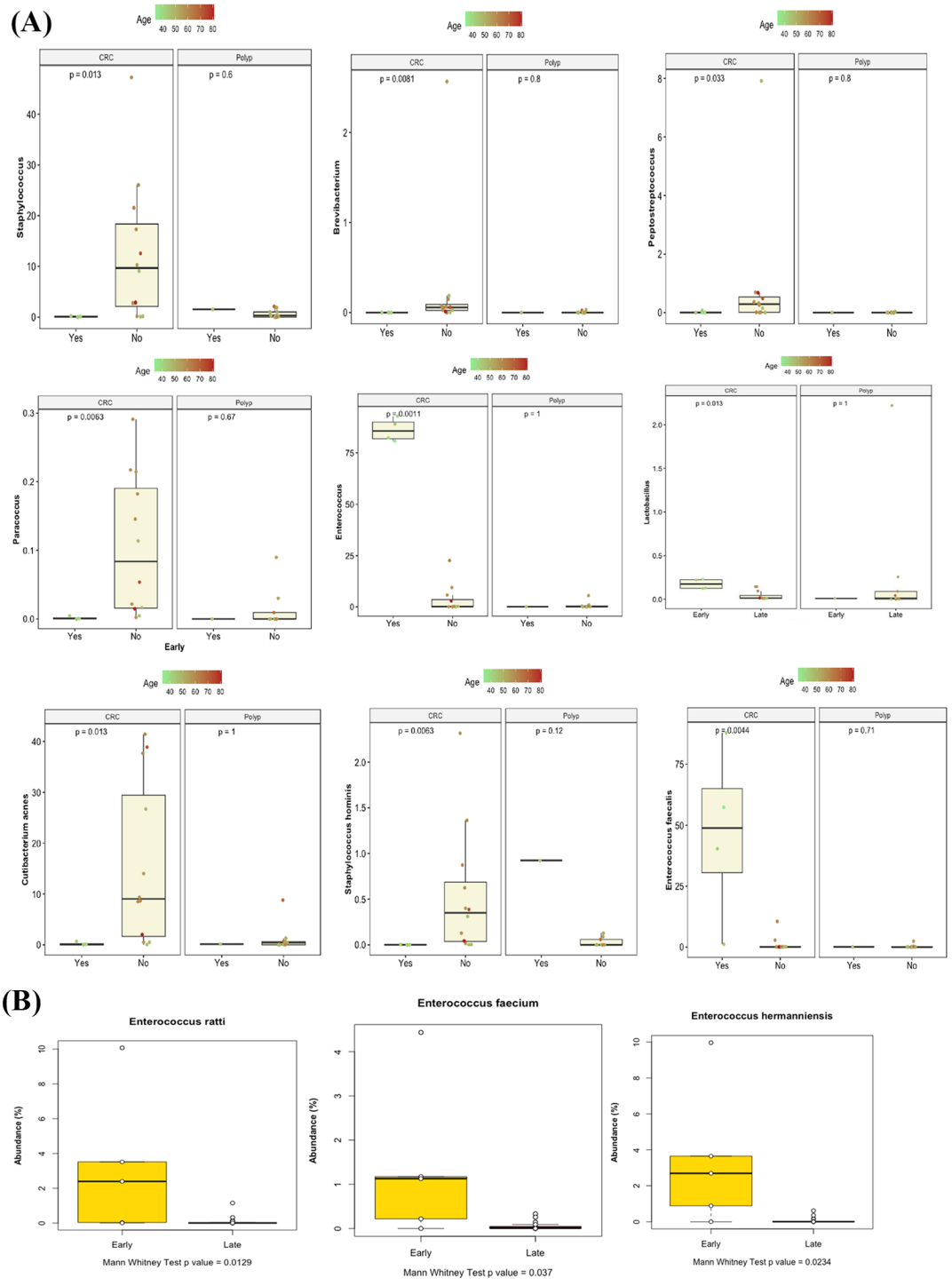

Fig. 7 (**A**) Taxon abundance at the genus level in CRC and colonic polyps groups split by age; (**B**) Taxon abundance at the species level in CRC and colonic polyps groups split by age: *p*-values were calculated by Mann-Whitney test; (Yes) refers to early onset, and (No) refers to late-onset

However, this study followed a targeted approach (real-time PCR on 16S rRNA genes of the phylum), which is unable to provide high-resolution taxonomic analysis.

Other studies from Egypt are all based on fecal microbiome profiling: A study by Elkholy et al. examined microbiome dysbiosis in patients with CRC from different ethnic groups, including Egyptians. It analyzed microbiome composition in CRC and normal tissue using short-read 16S rRNA sequencing. Distinct microbial signatures of Egyptian patients were reported compared to African American and European American patients. High abundance of *Herbaspirillum* and *Staphylococcus* was

reported in tumor tissues from Egyptian patients [16]. Additionally, an Egyptian pilot study used metagenomic sequencing and investigated gut microbiota in patients with CRC post-colectomy [17]. Another Egyptian study focused on ulcerative colitis patients, highlighting significant gut microbiome dysbiosis. That study demonstrated reduced anti-inflammatory bacteria in ulcerative colitis patients, such as *Firmicutes* and *Faecalibacterium prausnitzii* [18].

In the current work, we found that the bacterial families *Enterococcaceae, Enterobacteriaceae, Propionibacteriaceae*, and *Staphylococcaceae* were relatively more abundant in the CRC group, while *Bacteroidaceae, Morganellaceae, Lachnospiraceae, Yersiniaceae*, and *Erwiniaceae* were more abundant in colonic polyps. At the genus level, the most predominant bacterial genera with high OTUs in the CRC group were *Enterococcus, Cutibacterium, Staphylococcus, Corynebacterium*, and *Peptostreptococcus.* Other bacterial genera were also present in the CRC group but with lower relative abundance, e.g., *Dermabacter, Fusobacterium, Gulosibacter, Parvimonas, Proteus, Prevotella, Bacteroides*, and *Clostridium*. In addition, we found that *Proteus, Prevotella, Bacteroides,Macrococcus, Morganella, Mycolicibacter, Clostridium*, and *Lactobacillus* were significantly more abundant in the colonic polyps than in the CRC lesions.

A major finding here is that CRC lesions had a significantly higher relative abundance of the *Enterococcus* genus when compared with colonic polyps. In line with our findings, Wu et al. used 16S rRNA gene sequencing in previous research and demonstrated that the *Enterococcus* genus was relatively more abundant in patients with CRC than in the healthy controls [33]. In addition, Elahi et al., using TaqMan qPCR, also reported that *Enterococcus* was statistically significantly more abundant in CRC tissue samples [34]; however, TaqMan technology has lower resolution given its targeted nature; thus, confirmation of this finding by our long-read nanopore approach strengthens the results. Other studies agree with ours, by reporting a higher abundance of *Enterococcus* in stool samples from CRC patients than those from healthy controls [35, 36].

Enterococcus faecalis is thought to be a driver bacterium in CRC development through inducing inflammation and facilitating epigenetic changes and mutation accumulation [37]. We identified an elevated abundance of *Enterococcus faecalis* in the CRC group than in the colonic polyps group, which agrees with another study reported increased levels of *Enterococcus faecalis* in CRC patients [35]. *Enterococcus faecalis* was also reported to be associated with the onset and progression of CRC [31]. Our findings also propose its possible association with the early onset of CRC, although the data will need to be confirmed by multiple other studies. Previous studies reported that DNA-damaging superoxide radicals and genotoxins produced by *Enterococcus faecalis* may contribute to the CRC development [38, 39].

We also identified *Staphylococcus auricularis* as a prevalent bacterium in the CRC group. This bacterium was previously identified as one of the most common bacteria in healthy external auditory canal (EAC) culture [40], but it is not unusual the find of intraindividual divergence in microbiomes across the human body [41]. In addition, *Gulosibacter hominis* was identified in this study to be more abundant in CRC patients than in patients with colonic polyps. *Gulosibacter hominis* was earlier described as a unique source of opportunistic infections, the most common infections in persons with immunodeficiency [42]. Thus, we might postulate the relationship between this bacterium and a weakened immune system in CRC patients and disease development.

Our findings are in concordance with previously published data, by Osman et al., who reported an over-representation of *Peptostreptococcus stomatis*, *Fusobacterium nucleatum, Parvimonas micra*, and *Akkermansia muciniphila* in CRC patients when compared with non-CRC controls [43]. We noted the presence of the four formerly mentioned bacterial species in CRC lesions, which had higher relative abundance than in the colonic polyps. The CRC risk estimation analysis conducted using regional differences between Japan, China, the United States, Germany, France, and Austria revealed that *Peptostreptococcus stomatis* is a globally prevalent high-risk pathogen of CRC, and it is a significant variable in CRC risk prediction models worldwide [44]. Here, *Peptostreptococcus stomatis* was found to be much more prevalent in the CRC group. This finding agrees with previously published data from around the world, suggesting a potential role in CRC initiation [43–45]. Moreover, similar results were reported regarding the high abundance of *Peptostreptococcus stomatis* in CRC patients [46].

Strong clinical evidence suggests the association between *Fusobacterium nucleatum* and CRC [47]. It is well documented that *Fusobacterium* spp. are over-represented in CRC tumors, mainly *Fusobacterium nucleatum*, which was previously reported to have a critical role in CRC development [48, 49]. The gut microbiome of CRC patients differed from that of healthy controls, according to a recent study by Arafat et al., who used short-read 16S RNA sequencing to compare microbial diversity in mucosal samples of Kenyan CRC patients to that of healthy controls. Their analysis revealed that *Fusobacterium nucleatum* was present in high concentrations in all CRC patients compared with healthy individuals [50].

Another study matched with our findings identified *Fusobacterium* as CRC-enriched genera [51]. *Fusobacterium nucleatum* has been shown to enhance glycolysis

and promote oncogenesis in CRC by up regulating the expression of the lncRNA ENO1-IT1 [52]. It has been emerged also as a critical candidate for CRC predisposition due to its ability to bind to E-cadherin on the surface of colon cells via FadA adhesion, activating the Wnt/B-catenin signaling pathway and producing an inflammatory and oncogenic response, as well as its capacity to bind to the inhibitory immune receptor via Fap2 adhesin, altering natural killer cells [53]. The present study's findings agree with a large-scale meta-analysis from four cohorts of different ethnicities, using fecal samples' shotgun metagenomic sequencing, demonstrating abundance of *Parvimonas micra* in CRC patients over healthy controls [54]. Our findings also agree with previous study by Yu et al. that revealed significant higher abundance of *Fusobacterium nucleatum* and *Parvimonas micra* in feces of CRC patients compared to healthy controls [55].

Certain bacteria have shown a protective role against intestinal inflammation, such as *Bacteroides fragilis* [56, 57]. It was reported that polysaccharide A, the immunomodulatory molecule produced by *Bacteroides fragilis*, can induce an anti-inflammatory immune response to prevent intestinal inflammatory diseases in animals with colitis [58]. We found the relative abundance of *Bacteroides fragilis* to be lower in the CRC group, aligning with other studies [59, 60]. We also identified bacterial genera known to be protective against CRC, like *Clostridium* and *Lactobacillus.* Guo et al. reported that most *Clostridium* species have a possible beneficial role in preventing CRC by producing substances such as butyrate [61, 62]. For instance, the probiotic strains of *Lactobacillus* and *Bifidobacterium* were found to be at lower levels in patients with colorectal carcinoma. The protective role was suggested through their ability to secrete antibacterial peptides, compete for adhesion sites, and displace enteropathogens [63]. In addition, other studies revealed that *Lactobacillus* reduces gut inflammation. Such studies reported a significant reduction in the level of *Lactobacillus* in patient groups (polyps and CRC patients) compared with healthy controls [64, 65]. Despite the high translational potential of identifying CRC-protective bacterial species in treating and preventing CRC, research on it is still limited.

Conclusion

Our results revealed a considerable difference in the overall microbial diversity and the relative abundace of different bacterial taxa between colonic polyps and CRC lesions. Phylum Firmicutes and Actinomycetota were significantly abundant in the CRC group, while phylum Pseudomonadota and Bacteroidota were abundant in the colonic polyps group. The bacterial species *Enterococcus faecalis, Cutibacterium acnes, Peptostreptococcus stomatis, Fusobacterium nucleatum* were significantly enriched in the CRC group, while *Bacteroides fragilis, Proteus mirabilis*, and *Prevotella corporis* were more abundant in the colonic polyps group. Collectively, we demonstrated the microbial dysbiosis associated with CRC and colonic polyps groups. These findings provide a higher-resolution and more complete microbial profile of the cancerous and noncancerous tissue, which will lead to a better understanding of the pathogenesis of CRC, in general, and in Egyptian patients, in particular.

In addition, we provided initial clinical insights through identifying the microbiota associated with early- and late-onset CRC, as well as anatomical tumor site. The use of long-read nanopore sequencing offers a methodological improvement over previous studies. Future studies will investigate the metabolome profiles of these tissues and lesions to understand the impact of microbiome variations on cellular pathways. In addition, investigating the host-microbiome interaction, in animal models, is crucial to understand the causality and interaction between microbiome and colonic epithelium. Finally, exploring the use of prebiotics and probiotics as adjunctive CRC treatments is also being investigated by several research groups.

Limitations

Although this study supports our understanding of the tissue microbiome associated with CRC and colonic polyps in Egyptian patients, a larger sample size would provide a higher resolution and an ability to resolve subgroups based on tumor type, stage, as well as inter-individual differences. Multinational studies will enable a more comprehensive determination of the microbiota contributing to CRC development.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13099-025-00681-9.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

The authors thank the late Prof. Dr. Abdel-Rahman N. Zekri (deceased) for helping in manuscript conceptualization.

Author contributions

ASE-DY, AN, AAEL, and YMB: Conceptualization; ASE-DY, YMB, AN, AAEL, NMK, GNF, MTM, and AHM: Methodology; ASE-DY, AAEL, AN, and YMB: Resources; RKA, ASE-DY, AN, AAEL, and YMB: Data curation and Formal analysis; AN, AAEL, ASE-DY, and RKA: Writing - Original Draft; AAEL, AN, ASE-DY, RKA, and YMB: Review & Editing.

Funding sources

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The open access funding

for this research is provided by transformative agreement between Springer Nature and Science, Technology & Innovation Funding Authority (STDF) in cooperation with the Egyptian Knowledge Bank (EKB) agreement.

Data availability
All data generated or analyzed during this study and its supplementary information files are included in this article and any raw data can be obtained from the corresponding authors upon request.

Declarations

Ethics approval and consent to participate
The study protocol was accepted by the Institutional Review Board (IRB approval number: CB2309-302-071) of the National Cancer Institute (NCI), Cairo University, Egypt.

Competing interests
The authors declare no competing interests.

Author details
[1]College of Biotechnology, Misr University for Science and Technology, Giza, Egypt
[2]Virology and Immunology Unit, Cancer Biology Department, National Cancer Institute, Cairo University, Kasr Al-Aini st., Fom El-Khaleeg, Cairo 11976, Egypt
[3]Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Cairo, Egypt
[4]Center for Genome and Microbiome Research, Cairo University, Cairo, Egypt
[5]Cancer Biology Department, National Cancer Institute, Cairo University, Cairo, Egypt

Received: 7 August 2024 / Accepted: 23 January 2025
Published online: 02 February 2025

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70.
2. Barberis E, Joseph S, Amede E, Clavenna MG, La Vecchia M, Sculco M et al. A new method for investigating microbiota-produced small molecules in adenomatous polyps. Anal Chim Acta. 2021;1179.
3. Youssef ASED, Abdel-Fattah MA, Lotfy MM, Nassar A, Abouelhoda M, Touny AO et al. Multigene panel sequencing reveals Cancer-specific and common somatic mutations in Colorectal Cancer patients: an Egyptian experience. Curr Issues Mol Biol. 2022;44.
4. Wong CC, Yu J. Gut microbiota in colorectal cancer development and therapy. Nat Rev Clin Oncol. 2023.
5. Satam H, Joshi K, Mangrolia U, Waghoo S, Zaidi G, Rawool S, et al. Next-generation sequencing technology: current trends and advancements. Biology (Basel). 2023;12:997.
6. Chen Y, Nie F, Xie SQ, Zheng YF, Dai Q, Bray T et al. Efficient assembly of nanopore reads via highly accurate and intact error correction. Nat Commun. 2021;12.
7. Thomas AM, Manghi P, Asnicar F, Pasolli E, Armanini F, Zolfo M et al. Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation. Nat Med. 2019;25.
8. Gao R, Wu C, Zhu Y, Kong C, Zhu Y, Gao Y et al. Integrated Analysis of Colorectal Cancer reveals cross-cohort gut Microbial signatures and Associated serum metabolites. Gastroenterology. 2022;163.
9. He T, Cheng X, Xing C. The gut microbial diversity of colon cancer patients and the clinical significance. Bioengineered. 2021;12.
10. Mandal RS, Saha S, Das S. Metagenomic surveys of gut microbiota. Genomics Proteomics Bioinformatics; 2015.
11. Goodwin AC, Destefano Shields CE, Wu S, Huso DL, Wu XQ, Murray-Stewart TR et al. Polyamine catabolism contributes to enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis. Proc Natl Acad Sci U S A. 2011;108.
12. Rizkallah M, Saad R, Aziz R. The human Microbiome Project, Personalized Medicine and the birth of Pharmacomicrobiomics. Curr Pharmacogenomics Person Med. 2012;8.
13. Elrakaiby M, Dutilh BE, Rizkallah MR, Boleij A, Cole JN, Aziz RK. Pharmacomicrobiomics: the impact of human microbiome variations on systems pharmacology and personalized therapeutics. OMICS. 2014.
14. Picardo SL, Coburn B, Hansen AR. The microbiome and cancer for clinicians. Crit Rev Oncol Hematol. 2019.
15. El-Sokkary MMA. Molecular characterization of gut microbial structure and diversity associated with colorectal cancer patients in Egypt. Pan Afr Med J. 2022;43.
16. Elkholy A, Avuthu N, Abdalla M, Behring M, Bajpai P, Kim HG et al. Microbiome diversity in African American, European American, and Egyptian colorectal cancer patients. Heliyon. 2023;9.
17. Abo-Hammam RH, Salah M, Shabayek S, Hanora A, Zakeer S, Khattab RH. Metagenomic analysis of fecal samples in colorectal cancer egyptians patients post colectomy: a pilot study. AIMS Microbiol. 2024;10.
18. Ahmed EA, Ahmed SM, Zakaria NH, Baddour NM, Header DA. Study of the gut microbiome in Egyptian patients with active ulcerative colitis. Revista De Gastroenterología De México. (English Edition). 2023;88:246–55.
19. Elaskary S, Elgamal A, Badawy H, khalil M, Elgendy D, Elhagary H, et al. Gut microbiota analysis in colorectal diseased patients in Menoufia University Hospitals, Egypt. Microbes Infect Dis. 2024;0:0–0.
20. Liang Q, Chiu J, Chen Y, Huang Y, Higashimori A, Fang J et al. Fecal bacteria act as novel biomarkers for noninvasive diagnosis of colorectal cancer. Clin Cancer Res. 2017;23.
21. Yazici C, Wolf PG, Kim H, Cross TWL, Vermillion K, Carroll T et al. Race-dependent association of sulfidogenic bacteria with colorectal cancer. Gut. 2017;66.
22. Sánchez-Alcoholado L, Ramos-Molina B, Otero A, Laborda-Illanes A, Ordóñez R, Medina JA et al. The role of the gut microbiome in colorectal cancer development and therapy response. Cancers (Basel). 2020.
23. Zou S, Fang L, Lee MH. Dysbiosis of gut microbiota in promoting the development of colorectal cancer. Gastroenterol Rep (Oxf). 2018.
24. Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. Nat Rev Gastroenterol Hepatol. 2019.
25. Hua H, Sun Y, He X, Chen Y, Teng L, Lu C. Intestinal microbiota in colorectal adenoma-carcinoma sequence. Front Med (Lausanne). 2022;9.
26. Zhong X, Wang Y, Xu J, Cao H, Zhang F, Wang X. Gut microbiota signatures in tissues of the colorectal polyp and normal colorectal mucosa, and faeces. Front Cell Infect Microbiol. 2023;12.
27. Senthakumaran T, Moen AEF, Tannæs TM, Endres A, Brackmann SA, Rounge TB, et al. Microbial dynamics with CRC progression: a study of the mucosal microbiota at multiple sites in cancers, adenomatous polyps, and healthy controls. Eur J Clin Microbiol Infect Dis. 2023;42:305–22.
28. Wei P-L, Hung C-S, Kao Y-W, Lin Y-C, Lee C-Y, Chang T-H, et al. Classification of changes in the Fecal Microbiota Associated with Colonic adenomatous polyps using a long-read sequencing platform. Genes (Basel). 2020;11:1374.
29. Russo E, Bacci G, Chiellini C, Fagorzi C, Niccolai E, Taddei A et al. Preliminary comparison of oral and intestinal human microbiota in patients with colorectal cancer: a pilot study. Front Microbiol. 2018;8.
30. Bamola VD, Ghosh A, Kapardar RK, Lal B, Cheema S, Sarma P et al. Gut microbial diversity in health and disease: experience of healthy Indian subjects, and colon carcinoma and inflammatory bowel disease patients. Microb Ecol Health Dis. 2017;28.
31. Gao Z, Guo B, Gao R, Zhu Q, Qin H. Microbiota disbiosis is associated with colorectal cancer. Front Microbiol. 2015;6.
32. Quaglio AEV, Grillo TG, De Oliveira ECS, Di Stasi LC, Sassaki LY. Gut microbiota, inflammatory bowel disease and colorectal cancer. World J Gastroenterol. 2022.
33. Wu N, Yang X, Zhang R, Li J, Xiao X, Hu Y et al. Dysbiosis signature of fecal microbiota in Colorectal Cancer patients. Microb Ecol. 2013;66.
34. Elahi Z, Shariati A, Bostanghadiri N, Dadgar-Zankbar L, Razavi S, Norzaee S et al. Association of Lactobacillus, Firmicutes, Bifidobacterium, Clostridium, and Enterococcus with colorectal cancer in Iranian patients. Heliyon. 2023;9.
35. Balamurugan R, Rajendiran E, George S, Samuel GV, Ramakrishna BS. Real-time polymerase chain reaction quantification of specific butyrate-producing bacteria, Desulfovibrio and Enterococcus faecalis in the feces of patients with colorectal cancer. J Gastroenterol Hepatol (Australia). 2008;23.
36. Wang T, Cai G, Qiu Y, Fei N, Zhang M, Pang X et al. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. ISME J. 2012;6.
37. Wang X, Huycke MM. Extracellular superoxide production by Enterococcus faecalis promotes chromosomal instability in mammalian cells. Gastroenterology. 2007;132.

38. Huycke MM, Abrams V, Moore DR. Enterococcus faecalis produces extracellular superoxide and hydrogen peroxide that damages colonic epithelial cell DNA. Carcinogenesis. 2002;23.
39. Wang X, Allen TD, May RJ, Lightfoot S, Houchen CW, Huycke MM. Enterococcus faecalis induces aneuploidy and tetraploidy in colonic epithelial cells through a bystander effect. Cancer Res. 2008;68.
40. Sjövall A, Aho VTE, Hyyrynen T, Kinnari TJ, Auvinen P, Silvola J et al. Microbiome of the Healthy External Auditory Canal. Otology Neurotology. 2021;42.
41. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC et al. Topographical and temporal diversity of the human skin microbiome. Science (1979). 2009;324.
42. Vandamme P, Peeters C, Seth-Smith HMB, Graf L, Cnockaert M, Egli A et al. Gulosibacter hominis sp. nov.: a novel human microbiome bacterium that may cause opportunistic infections. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology. 2021;114.
43. Osman MA, Neoh H, min, Ab Mutalib NS, Chin SF, Mazlan L, Raja Ali RA et al. Parvimonas micra, Peptostreptococcus stomatis, Fusobacterium nucleatum and Akkermansia muciniphila as a four-bacteria biomarker panel of colorectal cancer. Sci Rep. 2021;11.
44. Shuwen H, Yinhang W, Xingming Z, Jing Z, Jinxin L, Wei W et al. Using whole-genome sequencing (WGS) to plot colorectal cancer-related gut microbiota in a population with varied geography. Gut Pathog. 2022;14.
45. Loftus M, Hassouneh SAD, Yooseph S. Bacterial community structure alterations within the colorectal cancer gut microbiome. BMC Microbiol. 2021;21.
46. Uchino Y, Goto Y, Konishi Y, Tanabe K, Toda H, Wada M, et al. Colorectal Cancer patients have four specific bacterial species in oral and gut microbiota in Common—A metagenomic comparison with healthy subjects. Cancers (Basel). 2021;13:3332.
47. Dey P, Chaudhuri SR. Cancer-Associated Microbiota: From Mechanisms of Disease Causation to Microbiota-Centric Anti-Cancer Approaches. Biology (Basel). 2022.
48. Wang N, Fang JY. Fusobacterium nucleatum, a key pathogenic factor and microbial biomarker for colorectal cancer. Trends Microbiol. 2023.
49. Vogtmann E, Hua X, Zeller G, Sunagawa S, Voigt AY, Hercog R et al. Colorectal cancer and the human gut microbiome: reproducibility with whole-genome shotgun sequencing. PLoS ONE. 2016;11.
50. Arafat W. P-316 Profile of Microbiota is associated with early onset of colorectal cancer in Egyptian and Kenyan patients. Ann Oncol. 2020;31.
51. Liu G, Li T, Zhu X, Zhang X, Wang J. An independent evaluation in a CRC patient cohort of microbiome 16S rRNA sequence analysis methods: OTU clustering, DADA2, and Deblur. Front Microbiol. 2023;14.
52. Hong J, Guo F, Lu S-Y, Shen C, Ma D, Zhang X, et al. *F. nucleatum* targets lncRNA ENO1-IT1 to promote glycolysis and oncogenesis in colorectal cancer. Gut. 2021;70:2123–37.
53. Rubinstein MR, Baik JE, Lagana SM, Han RP, Raab WJ, Sahoo D et al. Fusobacterium nucleatum promotes colorectal cancer by inducing Wnt/β-catenin modulator annexin A1. EMBO Rep. 2019;20.
54. Dai Z, Coker OO, Nakatsu G, Wu WKK, Zhao L, Chen Z et al. Multi-cohort analysis of colorectal cancer metagenome identified altered bacteria across populations and universal bacterial markers. Microbiome. 2018;6.
55. Yu J, Feng Q, Wong SH, Zhang D, Liang Q yi, Qin Y et al. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. Gut. 2017;66:70–8.
56. Jones SE, Paynich ML, Kearns DB, Knight KL. Protection from Intestinal inflammation by bacterial exopolysaccharides. J Immunol. 2014;192.
57. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008;453.
58. Round JL, Mazmanian SK. Inducible Foxp3 + regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010;107.
59. Lee YK, Mehrabian P, Boyajian S, Wu W-L, Selicha J, Vonderfecht S et al. The protective role of Bacteroides fragilis in a Murine Model of Colitis-Associated Colorectal Cancer. mSphere. 2018;3.
60. Chan JL, Wu S, Geis AL, Chan GV, Gomes TAM, Beck SE et al. Non-toxigenic Bacteroides fragilis (NTBF) administration reduces bacteria-driven chronic colitis and tumor development independent of polysaccharide A. Mucosal Immunol. 2019;12.
61. Guo Q, Goldenberg JZ, Humphrey C, El Dib R, Johnston BC. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database of Systematic Reviews; 2019.
62. Fukugaiti MH, Ignacio A, Fernandes MR, Ribeiro U, Nakano V, Avila-Campos MJ. High occurrence of fusobacterium nucleatum and clostridium difficile in the intestinal microbiota of colorectal carcinoma patients. Brazilian J Microbiol. 2015;46.
63. Tortora SC, Bodiwala VM, Quinn A, Martello LA, Vignesh S. Microbiome and colorectal carcinogenesis: linked mechanisms and racial differences. World J Gastrointest Oncol. 2022;14.
64. Fang CY, Chen JS, Hsu BM, Hussain B, Rathod J, Lee KH. Colorectal cancer stage-specific fecal bacterial community fingerprinting of the Taiwanese population and underpinning of potential taxonomic biomarkers. Microorganisms. 2021;9.
65. Mira-Pascual L, Cabrera-Rubio R, Ocon S, Costales P, Parra A, Suarez A et al. Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers. J Gastroenterol. 2015;50.

Publisher's note