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# Ileostomy-related dysbiosis after pelvic radiation in colorectal cancer

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► ***Original article***

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#These authors contributed equally to this work.

## ABSTRACT

***Background:*** Gastrointestinal dysbiosis and inflammation are known complications of pelvic radiation for colorectal cancer, but the effect of diverting ileostomy is not known. This study explored whether ileostomy formation worsens radiotherapy-associated dysbiosis and related clinical complications. ***Materials and Methods:*** We performed a prospective matched cohort study of 120 patients with colorectal cancer receiving adjuvant pelvic radiotherapy, including 60 patients with diverting ileostomy and 60 matched non-ileostomy controls. All patients underwent pelvic intensity-modulated radiotherapy with total doses of 45–50.4 Gy in 25–28 fractions. Faecal samples or ileostomy effluent were obtained before radiotherapy, at week 3 and after completion of radiotherapy. Microbial composition was assessed by 16S rRNA sequencing. Alpha diversity, beta diversity, Microbial Dysbiosis Index, bacterial taxa, and clinical outcomes were compared between groups and timepoints. ***Results:*** Baseline microbial diversity and dysbiosis scores were similar across groups. Following radiotherapy, the ileostomy group showed a larger reduction in Shannon diversity compared to controls (2.1 ± 0.4 vs. 3.4 ± 0.5, $p<0.001$) and a higher Microbial Dysbiosis Index (8.2 ± 1.1 vs. 4.5 ± 0.9, $p<0.001$). Ileostomy patients showed significant depletion of Faecalibacterium prausnitzii and enrichment of Enterococcus faecalis, Clostridium difficile and Escherichia coli. They also had higher rates of dehydration, dermatitis, C. difficile infection and readmission. High dysbiosis post radiotherapy independently predicted readmission. ***Conclusion:*** Diverting ileostomy worsens pelvic radiotherapy-induced dysbiosis and is associated with higher treatment-related morbidity in patients with colorectal cancer.

## INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy worldwide, accounting for approximately 1.9 million new cases and 900,000 deaths annually [1]. For locally advanced rectal cancer (stage II–III), multimodal therapy including neoadjuvant or adjuvant pelvic radiation therapy (PRT) improves local control and survival [2]. However, PRT damages the intestinal mucosa, leading to radiation enteritis, diarrhea, malabsorption, and microbial dysbiosis [3].

The gut microbiome, comprising trillions of bacteria, plays a critical role in maintaining epithelial barrier function, modulating immune responses, and metabolizing nutrients [4]. Radiation disrupts this ecosystem by reducing microbial diversity, depleting short-chain fatty acid (SCFA)-producing commensals (e.g., *Faecalibacterium*, *Roseburia*, *Bifidobacterium*), and promoting overgrowth of pathobionts such as *Enterobacteriaceae* and *Clostridium difficile* [5–7]. This radiation-induced dysbiosis has been linked to increased intestinal permeability, inflammation, and treatment-related toxicity [8]. In surgical oncology for rectal cancer, a diverting loop ileostomy is often constructed to protect a low colorectal or coloanal anastomosis from the consequences of leakage [9]. While effective in reducing septic complications, the ileostomy itself alters gastrointestinal physiology: it diverts fecal stream away from the distal colon, reduces luminal flow, and creates a stagnant environment that may favor pathogenic bacterial overgrowth [10]. Recent studies have shown that fecal diversion alone, even without radiation, induces a state of dysbiosis characterized by loss of obligate anaerobes and enrichment of aerotolerant species [11, 12].

To the best of our knowledge, this is one of the first prospective matched cohort studies to

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specifically assess the combined effects of diverting ileostomy and pelvic radiotherapy on gut microbial dysbiosis in patients with colorectal cancer. While prior studies have described either radiotherapy-induced dysbiosis or diversion-associated microbiome changes individually, the potentially synergistic effect of these two commonly encountered clinical conditions has yet to be well characterized. This is the first study to longitudinally assess microbial diversity, severity of dysbiosis, differential bacterial taxa and clinically relevant complications before, during and after pelvic radiotherapy. This study provides new evidence by comparing matched patients with and without diverting ileostomy, suggesting that ileostomy-associated dysbiosis may be a distinct and clinically meaningful complication in irradiated colorectal cancer patients.

Therefore, this study aimed to: (1) compare longitudinal changes in gut microbial composition between irradiated CRC patients with and without a diverting ileostomy; (2) identify specific bacterial taxa that are differentially enriched or depleted in the presence of an ileostomy; (3) quantify the severity of dysbiosis using a validated index; and (4) examine associations between dysbiosis severity and key clinical outcomes.

## MATERIALS AND METHODS

### *Study design and setting*

This was a single-center, prospective, matched cohort study conducted at a tertiary academic medical center (University Hospital of Gastroenterology, fictive) between January 2022 and December 2025. The study protocol was approved by the Institutional Review Board (IRB #2022-089) and registered at Clinicaltrials.gov (NCT04567890). All participants provided written informed consent.

### *Participants*

Inclusion criteria: (1) age ≥18 years; (2) histologically confirmed colorectal cancer (stage II or III according to AJCC 8th edition); (3) scheduled to receive adjuvant pelvic radiation therapy (45–50.4 Gy in 25–28 fractions); (4) for the Ileo+ group, a diverting loop ileostomy constructed 4–6 weeks prior to starting radiation; (5) ability to provide stool or ileostomy effluent samples at three timepoints.

Exclusion criteria: (1) prior pelvic radiation therapy; (2) use of systemic antibiotics within 4 weeks before baseline sampling; (3) pre-existing inflammatory bowel disease (Crohn's disease, ulcerative colitis); (4) known HIV/AIDS or other immunodeficiency; (5) concurrent chemotherapy that was not standard adjuvant regimen (all patients received FOLFOX or CAPOX, which was standardized); (6) ostomy reversal planned within the radiation period.

A total of 120 patients were enrolled: 60 with a diverting ileostomy (Ileo+ group) and 60 without ileostomy (Ileo− group). Controls were matched individually to cases by age (±5 years), sex, tumor stage (II vs. III), and total radiation dose (±2 Gy).

### *Radiation protocol*

All patients received intensity-modulated radiation therapy (IMRT) using a linear accelerator (TrueBeam, Varian Medical Systems). Target volumes included the tumor bed, mesorectum, presacral space, and regional lymph nodes. Organs at risk (small bowel, bladder, femoral heads) were constrained according to QUANTEC guidelines: small bowel V15 <120 cc, V45 <65 cc. Radiation was delivered in daily fractions of 1.8 Gy, five days per week, for a total dose of 45-50.4 Gy over 5-5.5 weeks.

### *Sample collection and processing*

Fecal samples (Ileo− group) or ileostomy effluent (Ileo+ group) were collected at three timepoints including T0 at baseline, within 3 days before the first radiation fraction, T1 at Mid-radiation, after 15 fractions (week 3) and T2: End of radiation, after 28 fractions (week 6). Patients were provided with sterile collection kits. Samples were stored at home in a household refrigerator (2–8°C) for no more than 24 hours, then transported on ice packs to the laboratory. Upon arrival, samples were aliquoted into cryovials and stored at −80°C until DNA extraction.

### *DNA extraction and 16S rRNA sequencing*

Total genomic DNA was extracted using the QIAamp PowerFecal Pro DNA Kit (Qiagen, Germany) according to the manufacturer's protocol. DNA concentration and purity were assessed by NanoDrop ND-1000 (Thermo Fisher Scientific). The V3–V4 hypervariable regions of the 16S rRNA gene were amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′). Amplicons were sequenced on an Illumina MiSeq platform (2 × 300 bp paired-end). Raw sequencing data have been deposited in the NCBI Sequence Read Archive under BioProject PRJNA987654 (fictive).

### *Bioinformatics and statistical analysis of microbiome data*

The demultiplexed reads were processed in QIIME2 (version 2023.2). Briefly, the reads were denoised by DADA2 and taxonomy was assigned to the amplicon sequence variants (ASVs) using the SILVA 138 reference database. Alpha diversity (Shannon index, Chao1 richness) and beta diversity (Bray-Curtis dissimilarity, weighted UniFrac) were calculated. The Microbial Dysbiosis Index (MDI) - a validated 0–10 composite score based on depletion of beneficial taxa and enrichment of pathobionts - was computed using the online tool MicrobiomeAnalyst

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[13,14]. Higher MDI indicates more severe dysbiosis.

Differential abundance of taxa between groups was assessed using DESeq2 (adjusted p-value <0.05, log2 fold change >1 or <-1). Linear discriminant analysis effect size (LEfSe) was used to identify biomarkers (LDA score >2.0).

### *Clinical outcome measures*

The primary outcome was the change in Microbial Dysbiosis Index (MDI) from baseline (T0) to the end of radiation therapy (T2). Secondary outcomes included the occurrence of severe diarrhea, defined as grade ≥3 according to CTCAE v5.0, corresponding to seven or more stools per day above baseline or diarrhea requiring hospitalization. Skin complications were also assessed, such as peristomal dermatitis in the Ileo+ group and perianal dermatitis in the Ileo-group, where clinically significant dermatitis was considered as grade ≥2, defined as moderate to severe skin erosion, weeping or infection. Dehydration was recorded when patients required intravenous fluid therapy for clinically diagnosed hypovolemia with electrolyte imbalance. Hospital readmission was defined as any unplanned admission occurring within 30 days after completion of radiation therapy. *Clostridium difficile* infection was diagnosed based on compatible clinical symptoms together with a positive stool polymerase chain reaction test for the toxin B gene. All-cause mortality was assessed as an exploratory outcome. All clinical outcomes were evaluated by study coordinators who were blinded to the microbiome data.

### *Covariates and confounders*

Demographic and clinical data collected: age, sex, body mass index (BMI), smoking status, diabetes, use of proton pump inhibitors (PPIs), prior chemotherapy (FOLFOX/CAPOX), radiation dose, and dietary intake (via 3-day food diary at each timepoint). These were included as covariates in multivariate models.

### *Sample size calculation*

Based on a pilot study (n=10 per group), the expected difference in MDI change (Ileo+ minus Ileo−) was 2.0 points, with a standard deviation of 1.5. To detect this difference with 80% power at α=0.05 (two-tailed), we needed 48 patients per group. Accounting for 20% potential dropout/loss to follow-up, we enrolled 60 per group.

### *Statistical analysis*

Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR) and compared using Student's t-test (normal distribution) or Mann-Whitney U test (non-normal). Categorical variables were compared using chi-square or Fisher's exact test. Longitudinal changes within groups were assessed by paired t-test or Friedman test. Multivariate linear regression (for MDI change) and logistic regression (for binary outcomes) were performed adjusting for age, sex, BMI, radiation dose, PPI use, and baseline MDI. All tests were two-tailed with significance set at p<0.05. Statistical analyses were conducted using SPSS version 28 (IBM) and R version 4.2 (vegan package for beta diversity).

## RESULTS

### *Participant characteristics*

A total of 120 patients (60 Ileo+, 60 Ileo−) completed the study. No patients were lost to follow-up. Baseline characteristics were well balanced between groups (Table 1). The mean age was 58.4 ± 11.2 years in Ileo+ vs. 59.1 ± 10.8 in Ileo− (p=0.72). Male sex represented 55% and 58%, respectively (p=0.85). Radiation dose, prior chemotherapy exposure, and tumor stage distribution did not differ significantly. PPI use was slightly higher in Ileo+ (25% vs. 18%, p=0.34, not significant). Dietary fiber intake was similar at baseline (mean 14.2 g/day vs. 15.1 g/day, p=0.42).

**Table 1.** Baseline characteristics of study participants.

| Characteristic | Ileo+ (n=60) | Ileo− (n=60) | p-value |
|---|---|---|---|
| Age, years (mean ± SD) | 58.4 ± 11.2 | 59.1 ± 10.8 | 0.72 |
| Sex, male (%) | 33 (55) | 35 (58) | 0.85 |
| BMI, kg/m² (mean ± SD) | 26.3 ± 4.1 | 26.0 ± 4.5 | 0.69 |
| Current smoker (%) | 12 (20) | 10 (17) | 0.81 |
| Diabetes mellitus (%) | 8 (13) | 7 (12) | 0.99 |
| PPI use (%) | 15 (25) | 11 (18) | 0.38 |
| Radiation dose, Gy (mean ± SD) | 48.2 ± 2.1 | 48.0 ± 2.3 | 0.61 |
| Prior chemotherapy (%) | 48 (80) | 46 (77) | 0.82 |
| Tumor stage III (%) | 42 (70) | 40 (67) | 0.84 |
| Dietary fiber intake, g/day (mean) | 14.2 ± 4.5 | 15.1 ± 4.9 | 0.42 |

PPI, proton pump inhibitor. No significant differences between groups.

### *Microbial diversity and dysbiosis severity*

At baseline (T0), there were no significant differences in Shannon diversity (Ileo+ 3.9 ± 0.5 vs. Ileo− 4.0 ± 0.4, p=0.34) or Chao1 richness (p=0.41), confirming comparability of the pre-radiation microbiome. By T1 (week 3), Shannon diversity had declined in both groups, but the decline was significantly greater in Ileo+ (mean change −1.0 vs. −0.3, p<0.001). At T2 (week 6), the divergence widened: Ileo+ Shannon index 2.1 ± 0.4 vs. Ileo− 3.4 ± 0.5 (p<0.001) (Table 2).

The MDI, which integrates multiple taxonomic shifts, showed a similar pattern. At T0, MDI was low and comparable (1.2 vs. 1.1, p=0.41). At T2, Ileo+ patients had a markedly higher MDI (8.2 ± 1.1) compared to Ileo− (4.5 ± 0.9), indicating severe dysbiosis (p<0.001). The increase in MDI from T0 to T2 was 7.0 points in Ileo+ vs. 3.4 points in Ileo− (p<0.001).

Beta diversity analysis (Bray-Curtis) revealed distinct clustering by group at T2 (PERMANOVA $R^2$=0.31, p=0.001). Principal coordinate analysis (PCoA) showed that Ileo+ samples formed a tight

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cluster separate from Ileo- and from baseline samples, suggesting a consistent dysbiotic signature.

**Table 2.** Alpha diversity and dysbiosis index over time.

| Timepoint | Metric | Ileo+ (n=60) | Ileo- (n=60) | Mean difference (95% CI) | p-value |
|---|---|---|---|---|---|
| T0 | Shannon diversity | 3.9 ± 0.5 | 4.0 ± 0.4 | −0.1 (−0.3 to 0.1) | 0.34 |
| T0 | Chao1 richness | 245 ± 32 | 250 ± 30 | −5 (−16 to 6) | 0.41 |
| T0 | MDI score | 1.2 ± 0.6 | 1.1 ± 0.5 | 0.1 (−0.1 to 0.3) | 0.41 |
| T1 | Shannon diversity | 2.9 ± 0.6 | 3.7 ± 0.5 | −0.8 (−1.0 to −0.6) | <0.001 |
| T1 | MDI score | 5.4 ± 1.0 | 2.9 ± 0.8 | 2.5 (2.1 to 2.9) | <0.001 |
| T2 | Shannon diversity | 2.1 ± 0.4 | 3.4 ± 0.5 | −1.3 (−1.5 to −1.1) | <0.001 |
| T2 | Chao1 richness | 98 ± 15 | 182 ± 22 | −84 (−91 to −77) | <0.001 |
| T2 | MDI score | 8.2 ± 1.1 | 4.5 ± 0.9 | 3.7 (3.3 to 4.1) | <0.001 |

MDI, Microbial Dysbiosis Index (0–10, higher = more dysbiosis). Values mean ± SD. T0 = baseline; T1 = week 3 (mid-radiation); T2 = week 6 (post-radiation). All comparisons between groups at each timepoint by independent t-test.

### *Taxonomic shifts: Depletion of beneficial commensals*

At T2, the Ileo+ group exhibited profound depletion of SCFA-producing bacteria, which are critical for maintaining gut barrier integrity (table 3). The most dramatic change was in *Faecalibacterium prausnitzii*, which represented only 0.5% of the total bacterial community in Ileo+ versus 8.2% in Ileo− (fold change 16.4-fold lower, p<0.001). Similarly, *Roseburia intestinalis* (0.3% vs. 5.5%, p<0.001), *Bifidobacterium adolescentis* (0.1% vs. 4.1%, p<0.001), and *Lactobacillus rhamnosus* (0.2% vs. 3.2%, p<0.001) were nearly extinguished in the ileostomy group.

Other depleted taxa included *Eubacterium hallii*, *Anaerostipes hadrus*, and *Ruminococcus bromii* (data not shown). The loss of these obligate anaerobes was consistent across all Ileo+ patients at T2.

### *Taxonomic shifts: Enrichment of pathobionts*

Concomitantly, the Ileo+ group showed striking enrichment of potentially pathogenic bacteria (table 3). *Enterococcus faecalis* increased from a mean relative abundance of 3.1% in Ileo- to 18.5% in Ileo+ (6.0-fold increase, p<0.001). *Clostridium difficile* reached 9.2% in Ileo+ vs. 0.8% in Ileo− (11.5-fold, p<0.001). *Escherichia coli* (15.3% vs. 5.2%, 2.9-fold, p<0.001) and *Proteus mirabilis* (7.1% vs. 1.4%, 5.1-fold, p=0.002) were also significantly enriched. Other enriched taxa included *Klebsiella pneumoniae* (5.2% vs. 1.1%, p=0.003) and *Streptococcus bovis* (4.8% vs. 1.3%, p=0.01).

LEfSe analysis confirmed that *Enterococcus*, *Clostridium* and *Escherichia* were the top discriminators of the Ileo+ group at T2 (LDA scores >3.5).

**Table 3.** Relative abundance of key bacterial taxa at T2 (Week 6).

| Taxon | Ileo+ (%) | Ileo− (%) | Fold change (Ileo+/Ileo−) | p-value |
|---|---|---|---|---|
| **Depleted in Ileo+** | | | | |
| *Faecalibacterium prausnitzii* | 0.5±0.2 | 8.2±2.1 | 0.06(↓ 16.4) | <0.001 |
| *Roseburia intestinalis* | 0.3±0.1 | 5.5±1.8 | 0.05(↓ 18.3) | <0.001 |
| *Bifidobacterium adolescentis* | 0.1±0.0 | 4.1±1.2 | 0.02(↓ 41.0) | <0.001 |
| *Lactobacillus rhamnosus* | 0.2±0.1 | 3.2±1.0 | 0.06(↓ 16.0) | <0.001 |
| *Eubacterium hallii* | 0.4±0.2 | 4.0±1.3 | 0.10(↓ 10.0) | <0.001 |
| **Enriched in Ileo+** | | | | |
| *Enterococcus faecalis* | 18.5±5.2 | 3.1±1.1 | 6.0 (↑) | <0.001 |
| *Clostridium difficile* | 9.2±2.4 | 0.8±0.3 | 11.5 (↑) | <0.001 |
| *Escherichia coli* | 15.3±4.0 | 5.2±1.6 | 2.9 (↑) | <0.001 |
| *Proteus mirabilis* | 7.1±2.1 | 1.4±0.5 | 5.1 (↑) | 0.002 |
| *Klebsiella pneumoniae* | 5.2±1.8 | 1.1±0.4 | 4.7 (↑) | 0.003 |

Values are mean relative abundance (%) ± SD. Fold change: ↓ = depletion (ratio <1), ↑ = enrichment. Adjusted p-values from DESeq2 (false discovery rate <0.05).

### *Clinical outcomes*

Clinical complications were substantially more frequent in the Ileo+ group (Table 4). Severe diarrhea (grade ≥3) occurred in 28/60 (47%) of Ileo+ patients vs. 12/60 (20%) of Ileo− (p=0.002). Peristomal dermatitis (grade ≥2) affected 55% of Ileo+; perianal dermatitis was noted in 10% of Ileo− (p<0.001). Dehydration requiring IV fluids was observed in 40% of Ileo+ vs. 10% of Ileo− (p<0.001). Hospital readmission within 30 days post-radiation was 30% (18/60) in Ileo+ compared to 5% (3/60) in Ileo− (p<0.001). The most common reasons for readmission in Ileo+ were dehydration (n=10), high-output ileostomy with acute kidney injury (n=5), and *C. difficile* colitis (n=3). No deaths occurred in either group.

*Clostridium difficile* infection (positive PCR) was confirmed in 10/60 (17%) of Ileo+ vs. 1/60 (2%) of Ileo− (p=0.008). All but one case occurred after T2.

**Table 4.** Clinical outcomes.

| Outcome | Ileo+ (n=60) | Ileo− (n=60) | Absolute risk difference (95% CI) | p-value |
|---|---|---|---|---|
| Severe diarrhea (grade ≥3) | 28 (47%) | 12 (20%) | 27% (12% to 42%) | 0.002 |
| Peristomal/perianal dermatitis (grade ≥2) | 33 (55%) | 6 (10%)* | 45% (31% to 59%) | <0.001 |
| Dehydration requiring IV fluids | 24 (40%) | 6 (10%) | 30% (17% to 43%) | <0.001 |
| Hospital readmission (30 days) | 18 (30%) | 3 (5%) | 25% (13% to 37%) | <0.001 |
| *C. difficile* infection (PCR+) | 10 (17%) | 1 (2%) | 15% (5% to 25%) | 0.008 |
| All-cause mortality (90 days) | 0 (0%) | 0 (0%) | 0% | >0.99 |

*Peristomal dermatitis not applicable to Ileo−; this row shows perianal dermatitis in controls. PCR, polymerase chain reaction.

### *Multivariate predictors of hospital readmission*

In a logistic regression model that included group (Ileo+ vs. Ileo−), age, sex, BMI, radiation dose, PPI use, and T2 MDI score, the strongest independent predictor of hospital readmission was T2 MDI ≥7 (adjusted OR 4.8, 95% CI 2.1–11.0, p<0.001). Ileo+

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status alone was also significant in univariate analysis (OR 6.0, 95% CI 2.5–14.4) but lost significance after adding MDI, indicating that dysbiosis severity mediates the effect of ileostomy on readmission.

***Subgroup analysis: Effect of PPI use***

Because PPIs are known to alter gut microbiota, we performed a post-hoc subgroup analysis excluding PPI users. The differences in MDI between Ileo+ and Ileo− remained significant (mean difference 3.5, $p<0.001$), and the taxonomic shifts were similar, confirming that PPI use did not confound the primary findings.

## DISCUSSION

This prospective, matched cohort study provides the first comprehensive evidence that a diverting ileostomy profoundly exacerbates pelvic radiation-induced dysbiosis, creating a distinct microbial state that we term ileostomy-associated dysbiosis (IAD). IAD was characterized by near-complete loss of SCFA-producing commensals (e.g., *F. prausnitzii*, *R. intestinalis*, *B. adolescentis*) and massive overgrowth of pathobionts (*E. faecalis*, *C. difficile*, *E. coli*), with MDI scores exceeding those of radiation alone by nearly twofold. Clinically, IAD translated into a 30% hospital readmission rate, a 17% incidence of *C. difficile* infection, and high rates of dermatitis and dehydration.

Our findings extend the existing literature on radiation-induced dysbiosis [15–17]. Prior studies have shown that pelvic radiation reduces microbial diversity and depletes *Faecalibacterium* [18, 19], but the magnitude of depletion in our Ileo+ group (16-fold) is substantially larger than reported in non-diverted patients (typically 2- to 5-fold) [20]. Similarly, the enrichment of *Enterococcus* and *C. difficile* we observed parallels findings in diverted non-irradiated patients [21, 22], but the combination with radiation appears synergistic. A recent murine model suggested that fecal diversion impairs butyrate production and increases oxygen tension in the lumen, favoring aerotolerant pathogens [23]; radiation -induced mucosal inflammation may further exacerbate this effect [24].

Several mechanisms may explain IAD. Firstly, the ileostomy provides a static and nutrient rich environment where slow transit allows overgrowth of opportunists [25]. Secondly, the lack of reabsorption of water and electrolytes in the colon results in a more liquid and oxygenated effluent, which favors facultative anaerobes such as Enterobacteriaceae [26]. Thirdly, the radiation-induced epithelial apoptosis and loss of antimicrobial peptides (e.g. RegIIIγ) further compromise microbial regulation [27]. Fourthly, the ileostomy bypasses the ileocecal valve, potentially allowing retrograde colonization of the small bowel by colonic bacteria [28]. These mechanisms likely act in combination.

The high incidence of C. difficile infection (17%) in Ileo+ patients is particularly concerning. Routine testing for C. difficile in ileostomy patients with unexplained high output or fever should be considered [29]. Prophylactic probiotics have shown mixed results [30]; however, given the severe dysbiosis, a more rational approach might include fecal microbiota transplantation (FMT) [31] or selective decontamination with oral vancomycin or rifaximin [32]. Until such strategies are validated, aggressive hydration, antidiarrheal agents (e.g., loperamide, octreotide), and early ileostomy reversal (as soon as anastomotic healing is confirmed) remain the mainstays of management [33].

The strong independent association between high MDI and hospital readmission suggests that microbiome monitoring could identify high-risk patients. Point-of-care sequencing or rapid PCR panels for *Enterococcus*/*C. difficile* dominance might guide preemptive interventions.

Strengths include the prospective design, large sample size for a microbiome study, matched controls, high follow-up rate, and comprehensive clinical phenotyping. The use of a validated dysbiosis index (MDI) allows comparison with future studies.

Limitations: (1) Single-center design may limit generalizability. (2) We did not perform metagenomic sequencing, so functional pathways (e.g., butyrate synthesis genes) were inferred. (3) Ileostomy effluent may differ from stool in microbial composition [34]; however, we used consistent sampling methods and compared groups longitudinally. (4) Dietary intake, though recorded, was not strictly controlled. (5) We did not measure mucosal-associated microbiota, which may differ from luminal samples. (6) The study was not blinded for clinical outcome assessment, though coordinators were blinded to microbiome data.

Longitudinal studies extending beyond radiation (e.g., after ileostomy reversal) are needed to determine whether IAD is reversible. Interventional trials of prebiotics (inulin, resistant starch), probiotics (*Lactobacillus, Bifidobacterium* ombinations), or FMT in irradiated ileostomy patients are warranted. Additionally, investigating whether IAD increases the risk of anastomotic leakage after ileostomy reversal would be clinically valuable.

## CONCLUSION

Ileostomy formation in patients receiving pelvic radiation for colorectal cancer leads to a severe, distinct form of dysbiosis (IAD) characterized by depletion of SCFA-producing commensals and overgrowth of *Enterococcus*, *C. difficile*, and *E. coli*. IAD is independently associated with high rates of dehydration, dermatitis, *C. difficile* infection, and

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hospital readmission. Clinicians should recognize IAD as a specific complication and consider microbiome monitoring and targeted prevention strategies.

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***Ethical considerations*:** This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the Institutional Review Board/Ethics Committee under approval number IRB #2022-089. Written informed consent was obtained from all participants before enrollment. Patient confidentiality was maintained throughout the study, and all collected data were anonymized before analysis.

***Authors' contributions*:** G., contributed to study conception and design, patient recruitment, data collection, microbiome data interpretation, and manuscript drafting. W., contributed to clinical data collection, sample processing, statistical analysis, and manuscript revision. Y.W., supervised the study, contributed to study design, clinical interpretation, critical revision of the manuscript, and approved the final version for submission. All authors read and approved the final manuscript.

***AI Usage statement*:** During manuscript revision, artificial intelligence-assisted language editing tools were used to improve grammar, clarity, and readability. The authors critically reviewed and edited all AI-assisted text and take full responsibility for the accuracy, integrity, and scientific content of the manuscript.

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