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Gut microbiome dysbiosis and colorectal cancer in low- and middle-income countries and the United States: A comparative analysis

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Abstract

Colorectal cancer (CRC) is a major cause of cancer deaths globally, with rising cases in low- and middle-income countries (LMICs). These trends have been associated with socioeconomic and lifestyle changes accompanying urbanization and dietary Westernization. The gut microbiome has emerged as a key mediator linking environmental factors to CRC. Microbial dysbiosis has been associated with a myriad of downstream carcinogenic effects through metabolic, immune, and inflammatory pathways. However, most existing research on microbiome-related CRC risk focuses on Western populations, creating a gap in comparative characterization of CRC-associated microbiomes in LMIC populations. To fill this gap, the study analyzed publicly available 16S ribosomal RNA (rRNA) sequencing datasets from Sri Lanka, Kenya, and the United States. We performed analyses in R and Python, including taxonomic profiling and alpha- and beta-diversity assessments. The results showed a consistently higher relative abundance of Fusobacteria in CRC than in controls across all populations (8.19% vs 1.64%). Alpha diversity analysis showed slightly higher microbial diversity in controls than in CRC samples, although Wilcoxon tests found no significant within-population differences, while Kruskal–Wallis and Dunn's tests identified significant population-level differences. Among CRC samples, United States (U.S.) cohorts exhibited the highest median Shannon diversity, followed by Kenya and Sri Lanka. Beta diversity showed substantial overlap among the U.S. cohorts, whereas Sri Lankan and Kenyan samples showed greater dispersion, indicating more variability. However, significant differences in multivariate dispersion (permutational analysis of multivariate dispersion, $p = 0.006$) indicated that differences in within-group heterogeneity also contributed to the observed separation. These findings highlight substantial microbial variability observed across populations, consistent with environment and lifestyle changes previously reported in transitioning populations. This research addresses a gap in the comparative characterization of CRC-associated microbiomes in underprivileged LMICs and provides a basis for future research evaluating environmental, dietary, and clinical factors.

Keywords: Colorectal cancer; Gut microbiome; 16S ribosomal RNA; Microbial diversity; Fusobacteria; Low- and middle-income countries

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1. Introduction

Colorectal cancer (CRC) is the uninhibited growth of cancerous cells in the large intestine, including the colon and rectum. It ranks as the third most frequently diagnosed cancer, following lung and breast cancer, and stands as one of the leading causes of cancer-related deaths among both genders in the U.S.[1,2] Globally, CRC accounts for 10% of cancers and is the second deadliest cancer.[3] In 2026, the United States (U.S.) alone is estimated to see ~260,000 new cases and 55,230 deaths.[2] CRC is a multifactorial disease that develops through accumulation of genetic and epigenetic alterations that disrupt normal regulation of intestinal epithelial cell growth and differentiation, ultimately leading to carcinoma.[4] These alterations may be inherited through germline mutations that confer a predisposition to CRC or acquired during an individual's lifetime, contributing to the development of nonfamilial or sporadic CRC. Although hereditary syndromes such as familial adenomatous polyposis and Lynch syndrome account for a proportion of CRC cases, most CRC cases are sporadic and are associated with multiple lifestyle and metabolic factors, including Western patterns, obesity, physical inactivity, and consumption of alcohol and processed foods.[5] At the molecular level, CRC involves alteration of genes that regulate cell proliferation, apoptosis, DNA repair, and signaling pathways. Mutations in tumor-suppressor genes such as *APC* and *TP53* and oncogenes such as *KRAS* can promote uncontrolled cellular proliferation and tumor progression.[4,6] Disruption of *APC*-mediated regulation of the Wingless-related integration site/β-catenin signaling pathway is an important early event in colorectal tumorigenesis, whereas subsequent alterations involving pathways such as mitogen-activated protein kinase, phosphoinositide 3-kinase/protein kinase B, and transforming growth factor-β can also contribute to disease progression.[6] These genetic and epigenetic alterations highlight the complex interplay between inherited susceptibility and acquired molecular changes in CRC development.

Geographically, CRC incidence varies substantially across regions worldwide. According to Global Cancer Observatory 2024 estimates, incidence is highest in Europe, Australia/New Zealand, and North America, whereas substantially lower rates are reported in regions including Eastern, Western, and Middle Africa and South-Central Asia.[7] Incidence is also up to four times higher in countries with a higher Human Development Index, highlighting the relationship between CRC and socioeconomic development and the epidemiological transition, as countries undergoing economic development experience changes in dietary patterns, physical activity, obesity, and other lifestyle-related risk factors.[5,7,8] Although CRC incidence has historically been higher in Western countries, recent decades have seen a rapid rise in incidence across low- and middle-income countries (LMICs) undergoing economic development and epidemiological transition, particularly among younger populations.[5,9] This shift has been strongly associated with urbanization and dietary westernization, in which traditional plant-based, high-fiber diets are increasingly replaced with diets high in fat, animal protein, and processed foods.[9,10] These changes have been hypothesized as potential contributors to the increasing burden of CRC in transitioning populations.

The gut microbiome has emerged as a critical mediator linking environmental exposures, particularly diet, to CRC risk.[11,12] Dysbiosis in the gut microbiome has been consistently associated with colorectal carcinogenesis. Gut microbiome communities contribute to CRC development through pathways such as production of genotoxic metabolites, modulation of immune responses, and altered bile acid metabolism.[13-15] For instance, the altered metabolomic profiles in CRC are characterized by increased levels of amino acids such as valine and phenylalanine and decreased levels of short-chain fatty acids, which are essential for maintaining intestinal integrity and health.[16-18] Transitional populations, especially those transitioning from traditional rural to urban lifestyles, offer a unique context for studying these interactions. These populations undergo rapid changes in diet, environment, and disease burden, enabling real-time observation of how microbiome shifts are associated with increased CRC risk.[19] For example, recent studies of African rural and urban populations have shown that traditional rural lifestyles support a more diverse and potentially more resilient gut microbiome than the more homogenized microbiomes found in urban settings.[10,20,21] Higher rates of CRC in urban and socioeconomically transitioning regions have been largely linked to dietary westernization and lifestyle changes.[10,22] Evidence suggests that the CRC-associated microbiome in developing countries closely resembles that of developed countries, and that microbiome composition can shift rapidly in response to dietary changes, thereby influencing cancer-related biomarkers.[15,23] Together, these findings indicate that microbial alterations associated with CRC may be conserved across populations, transcending regional, dietary, and socioeconomic differences. Although CRC incidence is increasing rapidly in LMICs, most of what we know about microbiome-associated CRC risk largely comes from Western populations.[21] This creates a significant knowledge gap regarding whether observed gut microbial patterns are universally applicable or population-specific. Although gut microbiome communities contribute to CRC

development through various metabolic activities, whether they contribute similarly in transitional populations remains insufficiently characterized.[3,10,15,20,24] Existing studies are often limited by cross-sectional designs, small sample sizes, and methodological variability, which further complicate interpretation.[20,25] A clear understanding of microbiome alterations in these transitional populations is necessary for early intervention, prevention, and development of targeted screening strategies. This gap is particularly concerning given the limited healthcare infrastructure and screening programs in many LMICs, where rising incidence directly translates into increased mortality.[19,26]

This study explores how changes in the gut microbiome are linked to CRC in populations undergoing urban transitions. It focuses on gut microbial patterns associated with CRC across populations, with particular attention to differences in microbial dysbiosis between non-Western groups undergoing transition and those with long-standing Western diets. Using a comparative, integrative analysis of publicly available sequencing data from Sri Lanka, Kenya, and the United States, the study investigates differences in microbial composition and diversity associated with CRC across populations. It provides insights into how CRC-associated microbial signatures vary across different urbanization levels. By disentangling disease effects from population background, this work aims to improve understanding of CRC-related dysbiosis, especially in populations transitioning away from traditional diets.

2. Materials and methods

2.1. Study design and data collection

A structured literature search was conducted to identify publicly available 16S ribosomal RNA (rRNA) sequencing datasets suitable for comparative analysis of CRC-associated mucosal microbiomes across populations. Only studies meeting the following inclusion criteria were selected: (i) publicly available raw paired-end FASTQ files in public repositories (National Center for Biotechnology Information–Sequence Read Archive [SRA] or European Molecular Biology Laboratory–European Bioinformatics Institute); (ii) tissue biopsy-derived samples from colorectal sites; (iii) use of 16S rRNA amplicon sequencing on Illumina platforms; (iv) clear clinical metadata, including disease status and demographic variables. We excluded antibiotic-exposed samples, as all source studies excluded individuals receiving antibiotics at the time of sampling. Based on these criteria, three research articles with data from Sri Lanka (SL) (PRJEB77293),[27] the U.S. (PRJNA986115),[28] and Kenya (K) (PRJNA986175)[29] were selected. Technical characteristics of the three source datasets, including the 16S rRNA target region, primer sequences, sequencing platform, read configuration, and sequencing facility, are summarized in Table S1. The SL and K cohorts each comprised 36 samples, including 18 CRC and 18 control (N) samples. The U.S. cohort comprised 36 African American (AA) samples (18 CRC and 18 N) and 38 White American (WA) samples (19 CRC and 19 N). The control samples in the U.S. cohort were corresponding normal-adjacent samples from the same patients and served as paired within-patient controls. Thus, the final analytical cohorts comprised 36 samples from SL, 36 samples from K, and 74 samples from the U.S., for a total of 146 samples. Because the source datasets did not measure dietary intake or individual-level dietary transition, we contextualized the role of dietary Westernization based on existing literature and did not measure it directly in the present study.

2.2. Data gathering and metadata construction

The raw paired-end FASTQ files corresponding to the selected BioProject accessions were downloaded using the SRA Toolkit (version 3.1.1, National Center for Biotechnology Information, U.S.) in Windows Command Prompt. The metadata table was constructed from information in the corresponding SRA Run Selector tables, BioProject entries, and associated published manuscripts. For each CRC and control sample, the following information was gathered when available: country, population background, clinical condition, age, gender, tumor site, BioProject and SRA run accession numbers, and sequencing instrumentation platform. No demographic or clinical variables were inferred unless they were clearly documented directly in the published study materials (Table S2).

2.3. DADA2: Sequence data processing and amplicon sequence variant inference

To ensure comparability across populations, a standardized bioinformatics workflow was followed to process the raw FASTQ files separately for each country. Sequence denoising and amplicon sequence variant (ASV) inference were performed separately for each country using the DADA2 pipeline (version 1.32.0) in R (version 4.4.2, R Foundation for Statistical Computing, Austria), following the standard DADA2 workflow.[30]

First, per-base quality profiles of the forward and reverse reads were inspected. The reads were then filtered and trimmed to remove low-quality regions. Then, error rates were learned independently for forward and reverse reads, followed by sequence denoising, merging of paired-end reads, and compilation into an ASV count table. Sequence lengths were then inspected to verify the

amplicon's consistency across samples. Chimeric sequences were identified and removed using the consensus method. Finally, read counts were tracked at each processing step to assess sequence retention and data quality. The resulting non-chimeric ASV tables were used for downstream taxonomic, diversity, and functional analyses. The cohort-specific sequencing depth, quality-control, and filtering parameters from DADA2 analysis are provided in Table S3.

2.4. Downstream analysis

2.4.1. Taxonomic assignment and phyloseq object construction

The ASV tables from the SL, K, and U.S. cohorts were merged into a unified feature table for downstream analyses. Taxonomic classification of ASVs was performed in DADA2 using a naive Bayes classifier trained on the SILVA reference database (release 138.2). The combined ASV table, taxonomic assignments, and corresponding sample metadata were integrated using the phyloseq package (version 1.48.0) in R to construct a comprehensive phyloseq object. Sample metadata was curated to ensure consistency of sample identifiers across datasets before integration. The resulting phyloseq object was used to visualize taxonomic composition and to perform microbial diversity analyses.

To visualize taxonomic classifications for each country, the phyloseq object was agglomerated at the phylum level. Cohorts were then subset by condition and merged by country to enable cohort-level comparisons. Relative abundances were calculated for each phylum within the merged samples. Taxonomic bar plots were generated using the ggplot2 package, showing phylum-level composition in CRC and control groups across cohorts.

2.4.2. Microbiome diversity analyses

Microbial diversity analyses were conducted in R using the phyloseq(), vegan(), and ggplot2() packages. For this study, the Alpha diversity metrics, including Shannon diversity,[31] Simpson diversity,[32] and observed ASV richness, were calculated for each CRC and control sample. The resulting metrics were merged with sample metadata and visualized as boxplots with jittered points, stratified by population and disease status.

For beta-diversity analysis, ASV counts were first transformed into relative abundances, and then Bray–Curtis dissimilarity matrices[33] were calculated. The community-level differences were then visualized using principal coordinates analysis (PCoA).

2.5. Statistical analysis

The alpha diversity was assessed using Shannon, Simpson, and observed richness indices calculated with phyloseq(). Differences between CRC and control within each population were evaluated using Wilcoxon rank-sum and Wilcoxon signed-rank tests. The differences among populations were assessed separately for CRC using Kruskal–Wallis tests, followed by Dunn's post-hoc pairwise comparison with Benjamini–Hochberg adjustments. All tests were performed with statistical significance defined at $p < 0.05$ for Wilcoxon and Kruskal–Wallis tests, and adjusted $p < 0.05$ for Dunn's post-hoc test.

The visual patterns from beta-diversity analysis were statistically evaluated using permutational multivariate analysis of variance (PERMANOVA). PERMANOVA was performed using the *adonis2()* function in the vegan package to evaluate differences in microbial community composition between CRC and control groups within each population, among CRC populations, and within U.S. CRC groups. To assess whether differences in within-group dispersion influenced significant PERMANOVA results, we conducted a permutational analysis of multivariate dispersion (PERMDISP) using the *betadisper()* and *permutest()* functions. All tests were performed with 999 permutations, and statistical significance was defined as $p < 0.05$.

3. Results

3.1. Gut microbial structure

Firmicutes, Bacteroidetes, Proteobacteria, and Fusobacteria comprised the majority of the microbiome in all groups, although their relative abundances varied between populations (Figure 1). Population-specific differences were clear: SL_CRC samples showed higher Proteobacteria, and K_CRC samples showed higher Bacteroidetes, relative to U.S. CRC samples.

In SL_CRC cases, Proteobacteria (32.4%) and Firmicutes (30.3%) were most abundant, followed by Bacteroidetes (22.6%). K_CRC individuals showed a higher proportion of Bacteroidetes (49.51%) and moderate levels of Firmicutes (27.07%), while Proteobacteria were less prevalent (20.93%). Among AA_CRC participants, Firmicutes predominated (42%), followed by Bacteroidetes (22.13%) and Fusobacteria (13.03%). In WA_CRC, Firmicutes (43.76%) also predominated, along with Bacteroidetes (28.6%) and Proteobacteria (12.28%). Table 1 summarizes the mean relative abundances of these phyla in both the CRC and control groups across all populations.

Comparisons between CRC and control groups revealed population-specific alterations in phylum abundance. SL_CRC populations showed higher Firmicutes (30.30% vs. 28.37%) and Bacteroidetes (22.60% vs. 18.36%) than

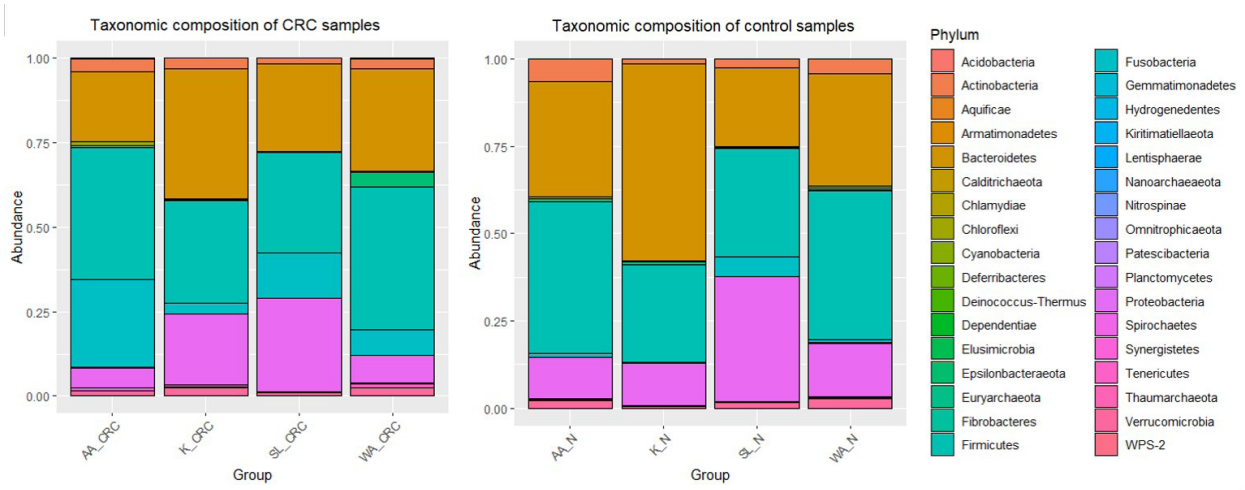

Figure 1. Phylum-level gut microbiome composition across study cohorts. Stacked bar charts illustrate the relative abundance of dominant bacterial phyla in colorectal cancer (CRC) and control groups from four population groups: Sri Lanka (SL_CRC, *n* = 18; SL_N, *n* = 18), Kenya (K_CRC, *n* = 18; K_N, *n* = 18), United States (U.S.) African American (AA) (USAA_CRC, *n* = 18; USAA_N, *n* = 18), and U.S. White American (WA) (USWA_CRC, *n* = 19; USWA_N, *n* = 19). Each bar shows the distribution of bacterial phyla within each group.

Table 1. Summary of the mean relative abundances of bacterial phyla in CRC and control samples across populations

Phylum	Mean relative abundance of CRC					Mean relative abundance of control				
	SL	K	AA	WA	CRC total (%)	SL	K	AA	WA	Control total (%)
Firmicutes	30.3	27.07	42	43.76	35.78	28.37	31.79	44.81	44.2	37.29
Bacteroidetes	22.6	49.51	22.13	28.6	30.71	18.36	52.57	26.56	28.62	31.52
Proteobacteria	32.4	20.93	8.79	12.28	18.6	44.12	11.12	14.2	17.85	21.83
Fusobacteria	11.55	4.52	13.03	3.63	8.19	4.15	0.14	1.28	0.99	1.64
Actinobacteria	1.78	2.2	5.72	3.74	3.36	2.72	2.11	6.43	3.87	3.78
Verrucomicrobiota	1.03	3.32	2.24	2.56	2.28	1.48	0.82	3.18	2.11	1.89
Other[a]	0.31	1.43	6.07	5.4	3.3	0.77	1.4	3.51	2.33	2

Notes: [a]Low-abundance phyla; "CRC Total (%)" and "Control Total (%)" represent the mean relative abundance across all study populations.
Abbreviations: AA: African American; CRC: Colorectal cancer; K: Kenya; SL: Sri Lanka; WA: White American.

controls, whereas Proteobacteria were less abundant in CRC (32.40% vs. 44.12%). Whereas Bacteroidetes were more prevalent in K control (52.57% vs. 49.51% in CRC), CRC samples had higher Proteobacteria (20.93% vs. 11.12%) and lower Firmicutes (27.07% vs. 31.79%). Among AA, Firmicutes levels were similar between CRC and control (42.00% vs. 44.81%), but Bacteroidetes (22.13% vs. 26.56%) and Proteobacteria (8.79% vs. 14.20%) were reduced in CRC. In WA participants, Firmicutes (43.76% vs. 44.20%) and Bacteroidetes (28.60% vs. 28.62%) were similar in CRC and control, and Proteobacteria were lower in CRC (12.28% vs. 17.85%).

Fusobacteria showed higher mean relative abundance in CRC than in controls across all studied populations: K (4.52% vs. 0.14%), SL (11.55% vs. 4.15%), AA (13.03% vs. 1.28%), and WA (3.63% vs. 0.99%). Across all populations, Fusobacteria abundance was approximately five-fold higher in CRC samples than in controls (8.19% vs. 1.64%). Actinobacteria were slightly less prevalent in CRC overall (mean 3.36% vs. 3.78% in control), while Verrucomicrobiota

showed similar mean relative abundances between CRC and control samples (2.28% vs. 1.89%). Distinct phylum-level patterns were also observed between the AA and WA CRC cohorts. Within the AA cohort, Spirochaetota (2.98% vs. 0.32%) and Cyanobacteria (1.4% vs. 0.64%) were more abundant in CRC than in controls. Similarly, Epsilonbacteraeota (3.29% vs. 0.59%) and Tenericutes (1.02% vs. 0.25%) showed higher mean relative abundance in WA CRC than in control samples. These differences may reflect variation in the microbial communities associated with CRC across the two U.S. cohorts.

Relative standard deviations (SD) reflected substantial variability within groups. Proteobacteria exhibited high dispersion, particularly in SL_CRC (SD = 34.37%) and K_CRC (SD = 28.68%) groups. Similarly, Fusobacteria showed considerable variability in the AA_CRC group (SD = 21.67%), as did Firmicutes in the WA_CRC group (SD = 11.58%). Similar variability was observed across other dominant phyla.

3.2. Gut microbiome diversity analysis

3.2.1. Alpha diversity

Alpha diversity was assessed using Shannon diversity, Simpson diversity, and observed richness to evaluate within-sample microbial diversity across CRC and control groups in the four populations. Figure 2 shows the distribution of Shannon diversity within each population.

The differences in alpha diversity were observed among populations: both AA and WA (Figure 2C and 2D) exhibited higher median Shannon diversity compared to the K (Figure 2B) and SL (Figure 2A) cohorts, with substantial overlap between CRC and control populations. The K_CRC group showed the lowest median diversity and a comparatively narrow distribution, suggesting reduced variation among individuals. The SL cohort showed the lowest overall diversity, with the CRC population also having reduced median diversity and greater variability than controls.

When comparing CRC and control groups within each population, controls generally showed slightly higher alpha diversity than CRC samples in the K and U.S. cohorts. However, the SL_CRC and control groups showed broadly similar distributions. We observed no statistically significant differences in alpha diversity between CRC and control samples within any population. In the SL and K cohorts, Wilcoxon rank-sum tests showed no significant differences in Shannon diversity, Simpson diversity, or observed richness (all $p > 0.05$). Similarly, paired Wilcoxon signed-rank tests in the African American and White American cohorts found no significant differences across the three alpha-diversity measures (all $p > 0.05$). Although descriptive differences in the distributions and median values were observed, none were statistically significant (Table S4).

To further assess population-level variation, CRC samples across the four populations were compared (Figure 3). A descriptive gradient emerged, with the U.S. cohorts generally showing the highest alpha diversity, followed by the K cohort, while the SL cohort showed the lowest diversity.

Kruskal–Wallis tests demonstrated significant differences among the four populations for all three alpha-diversity measures in the CRC group. Shannon diversity differed significantly among populations ($\chi^2 = 21.944$, df = 3, $p < 0.001$), as did Simpson diversity ($\chi^2 = 22.92$, df = 3, $p < 0.001$) and observed richness ($\chi^2 = 28.501$, df = 3, $p < 0.001$). Dunn's post-hoc pairwise comparisons with Benjamini–Hochberg adjustments were subsequently performed to identify specific population differences among CRC samples (Table 2). For Shannon diversity, SL_CRC samples had significantly lower diversity than both AA (adjusted $p = 0.001$) and WA (adjusted $p < 0.001$) samples. K_CRC samples also had significantly lower Shannon diversity than AA (adjusted $p = 0.018$) and WA (adjusted $p = 0.003$) samples. No significant differences were observed between AA and WA samples (adjusted $p = 0.561$), or K and SL samples (adjusted $p = 0.285$). For Simpson diversity, SL_CRC samples had significantly lower diversity than AA (adjusted $p = 0.0019$) and WA (adjusted $p < 0.001$) samples. K samples also had significantly lower Simpson diversity than AA (adjusted $p = 0.0087$) and WA (adjusted $p = 0.0022$) samples. The differences between K and SL samples (adjusted $p = 0.5932$) and between AA and WA samples (adjusted $p = 0.6767$) were not statistically significant. For observed richness, SL_CRC samples had significantly lower richness than AA (adjusted $p < 0.001$), K (adjusted $p = 0.001$), and WA samples (adjusted $p < 0.001$). No significant differences were observed between AA and K samples (adjusted $p = 0.286$), AA and WA samples (adjusted $p = 0.899$), or K and WA samples (adjusted $p =$ 0. 279).

3.2.2 Beta diversity

To examine shifts in the microbial community composition among CRC samples across populations, beta diversity analysis was performed using Bray–Curtis dissimilarities and visualized using PCoA (Figure 4). The first two principal coordinates (PCs) explained a modest proportion of the total variation (PC1 = 8.2%, PC2 = 6.2%), indicating substantial inter-individual variability within CRC-associated microbiomes. AA and WA

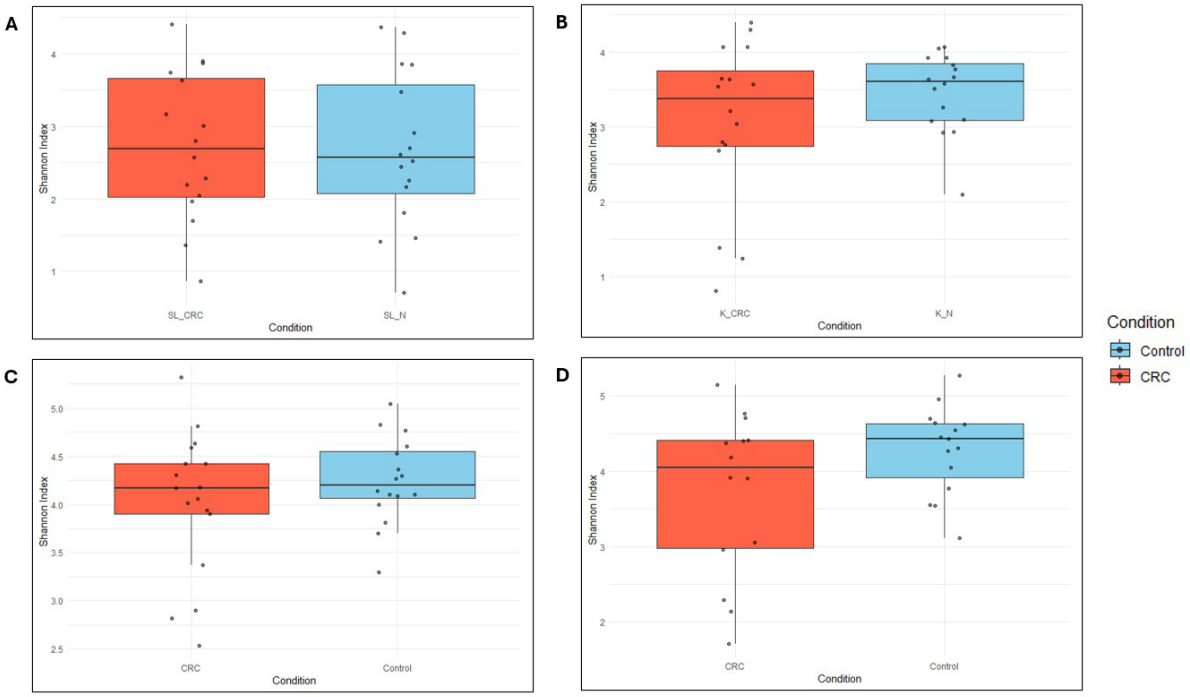

Figure 2. Alpha diversity within each cohort (colorectal cancer [CRC] vs. control). Boxplots illustrate the distribution of Shannon diversity values across cohorts. (A) Sri Lanka. (B) Kenya. (C) United States African American. (D) United States White American.

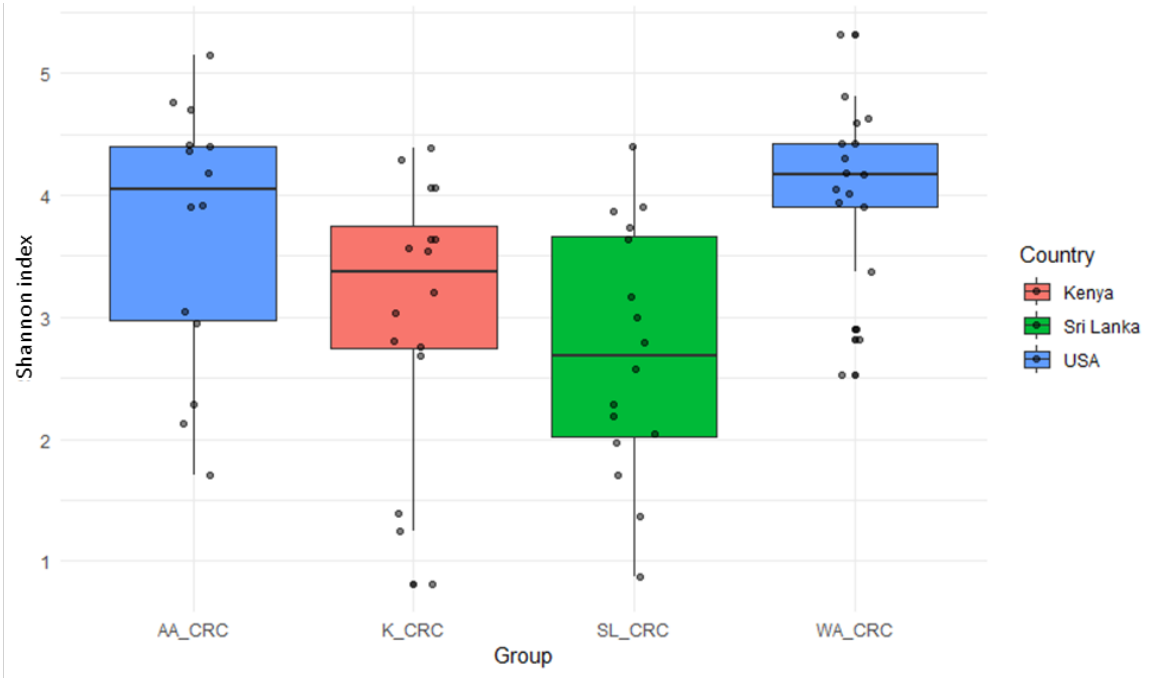

Figure 3. Alpha diversity among CRC cohorts across different populations. The Shannon diversity index was calculated using only CRC groups. Abbreviations: AA: African American; K: Kenya; SL: Sri Lanka; WA: White American.

Table 2. Dunn's post-hoc pairwise comparisons of colorectal cancer samples across the four populations

Measure	Comparison	*Z*	Unadjusted *p*	BH-adjusted *p*	Significant
Shannon	African American vs. Kenyan	2.349	0.018	0.028	Yes
Shannon	African American vs. Sri Lankan	3.417	<0.001	0.001	Yes
Shannon	Kenya vs. Sri Lanka	1.068	0.285	0.342	No
Shannon	African American vs. White American	−0.581	0.561	0.561	No
Shannon	Kenya vs. White American	−2.961	0.003	0.006	Yes
Shannon	Sri Lanka vs. White American	−4.044	<0.001	< 0.001	Yes
Simpson	African American vs Kenyan	2.757	0.005	0.008	Yes
Simpson	African American vs Sri Lankan	3.291	<0.001	0.001	Yes
Simpson	Kenya vs. Sri Lanka	0.534	0.593	0.593	No
Simpson	African American vs. White American	−0.577	0.563	0.676	No
Simpson	Kenya vs. White American	−3.371	<0.001	0.002	Yes
Simpson	Sri Lanka vs. White American	−3.912	<0.001	< 0.001	Yes
Observed	African American vs Kenyan	1.178	0.238	0.286	No
Observed	African American vs Sri Lankan	4.529	<0.001	< 0.001	Yes
Observed	Kenya vs. Sri Lanka	3.35	<0.001	0.001	Yes
Observed	African American vs. White American	−0.127	0.899	0.899	No
Observed	Kenya vs. White American	−1.321	0.186	0.279	No
Observed	Sri Lanka vs. White American	−4.716	<0.001	< 0.001	Yes

Abbreviation: BH: Benjamini–Hochberg.

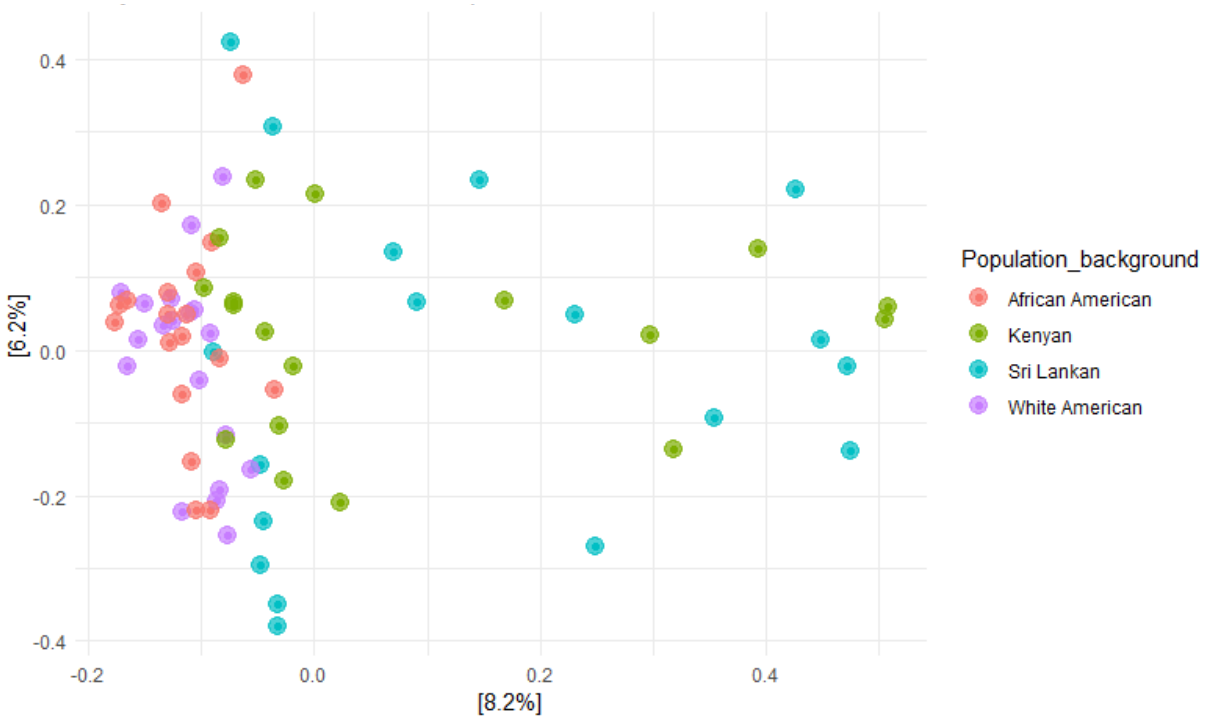

Figure 4. Beta diversity of colorectal cancer (CRC) samples across populations based on Bray–Curtis dissimilarity. Principal coordinates analysis of Bray–Curtis dissimilarities revealed substantial overlap among CRC samples from different populations, with differences in within-population dispersion.

largely overlapped, indicating broadly similar microbial community compositions within these two populations. K_CRC samples showed partial overlap with the U.S. CRC samples but were more widely distributed across the ordination, indicating greater heterogeneity in microbial community composition within the K cohort. Several K samples were separated from the U.S. cluster along both PC1 and PC2, suggesting the presence of population-specific microbial signatures. SL_CRC samples exhibited the greatest dispersion, particularly along PC1, and extended into regions of the ordination distinct from the U.S. and K samples. Although some overlap with the AA and WA CRC clusters was observed, the broader distribution of SL samples indicates increased within-population variability and potentially distinct microbiome configurations associated with CRC in this cohort.

Despite these trends, considerable overlap among populations was observed, indicating that CRC-associated microbiomes share common compositional features across geographic and ethnic groups while retaining population-specific variation. However, K_CRC and SL_CRC samples exhibited greater dispersion than AA and WA samples, suggesting increased heterogeneity within these cohorts. These visual patterns were statistically assessed using PERMANOVA (Table 3). Within-cohort analyses showed no significant differences in microbial community composition among SL_CRC samples (R^2 = 0.009, F = 0.326, p = 0.999) or U.S. CRC samples (R^2 = 0.013, F = 0.978, p = 0.509), consistent with the overlap observed in the PCoA. In contrast, the K population exhibited a significant within-cohort effect (R^2 = 0.052, F = 1.89, p = 0.001), consistent with increased dispersion noted in the ordination plot.

When restricting the analysis to CRC samples only, PERMANOVA identified a statistically significant link between population background and gut microbial community composition across SL, K, and the U.S. (R^2 = 0.077, F = 2.944, p = 0.001). No significant differences were found between AA and White CRC patients (R^2 = 0.024, F = 0.895, p = 0.852), which explains their close clustering in the PCoA plot.

To assess whether population-level differences were influenced by variability rather than centroid separation, a PERMDISP was performed (Table 4).

There was no significant within-cohort dispersion in SL_CRC, K_CRC, or U.S. CRC cohorts. However, significant differences in multivariate dispersion were also

Table 3. Permutational multivariate analysis of variance of population-level differences in CRC gut microbiome beta diversity (Bray–Curtis)

Analysis	Population/group	df	Sum of squares	R^2(effect size)	*F*	*p*-value
Within-cohort	Sri Lanka	1	0.126	0.009	0.326	0.999
	Kenya	1	0.743	0.052	1.89	0.001**
	U.S.	1	0.424	0.013	0.978	0.509
CRC-only	Sri Lanka vs. Kenya vs. U.S.	2	2.492	0.077	2.944	0.001**
U.S. CRC-only	AA vs. WA	1	0.396	0.024	0.895	0.852

Notes: R^2 represents the proportion of variance explained by each factor; *p*-values were obtained using permutation tests (999 permutations); $^{**}p \leq 0.001$. Abbreviations: AA: African American; CRC: Colorectal cancer; WA: White American.

Table 4. Permutational analysis of multivariate dispersion results for within-cohort and CRC-only comparisons

Analysis	Population/group	df	Sum of squares	Mean square	*F*	*p*-value
Within cohort	Sri Lanka	1	0.0048	0.0048	0.423	0.494
	Kenya	1	0.0059	0.0059	2.066	0.163
	U.S.	1	0.0025	0.0025	2.136	0.145
CRC-only	Sri Lanka vs. Kenya vs. U.S.	2	0.0311	0.0155	5.37	0.009 *
U.S. CRC-only	AA vs. WA	1	0.0002	0.0002	0.21	0.65

Notes: *p*-values were calculated using permutation tests (999 permutations) and assessed differences in multivariate dispersion among groups; $^{*}p < 0.05$. Abbreviations: AA: African American; CRC: Colorectal cancer; WA: White American.

observed ($F = 5.37$, $p = 0.009$), indicating differences in within-population dispersion which may contribute to the observed PERMANOVA result. No significant differences in dispersion were observed between AA_CRC and WA_CRC samples ($p = 0.65$).

4. Discussion

This study comparatively evaluated gut microbial composition and diversity in CRC patients and controls across South Asian (SL), U.S. (AA and WA), and K populations. At the phylum level, CRC-associated microbiomes retained a conserved core structure dominated by Bacteroidetes, Firmicutes, Fusobacteria, Actinobacteria, and Proteobacteria, consistent with previously reported gut microbial profiles in CRC.[34,35] Despite this shared taxonomic backbone, the relative abundances and ecological configurations of these phyla varied substantially across populations, indicating that CRC-associated dysbiosis appears to be shaped by population-specific microbial baselines rather than representing a universally uniform state.

Among U.S. cohorts, CRC-associated microbial profiles were broadly similar, likely reflecting shared dietary patterns and environmental exposures. Both AA_CRC and WA_CRC samples exhibited comparable Firmicutes dominance with mean relative abundances of 42% and 43.76%, respectively. Bacteroidetes were also relatively abundant in both groups, although their

abundance was higher in WA_CRC than in AA_CRC (28.60% vs. 22.13%). However, this similarity was not consistent across all taxa; Fusobacteria abundance differed markedly between the two groups (13.03% in AA_CRC vs. 3.63% in WA_CRC), while Proteobacteria displayed the opposite pattern, with 12.28% in WA_CRC vs. 8.79% in AA_CRC. These differences highlight that even within similar environments, specific CRC-associated taxa may exhibit population-level variability. SL_CRC and K_CRC samples displayed distinct microbial profiles, including markedly higher Proteobacteria in SL_CRC (32.40%) and higher Bacteroidetes in K_CRC (49.51%), consistent with the possibility that long-term dietary practices, early-life microbial exposures, and region-specific environmental factors shape baseline microbiome structure and influence CRC-associated microbial restructuring.[36,37]

Within-population comparisons between CRC and controls exhibited a higher relative abundance of Fusobacteria across all cohorts, supporting its role as a conserved microbial feature of CRC.[38,39] However, because the present study analyzed this taxon only at the phylum level, the observed pattern cannot be attributed specifically to *Fusobacterium nucleatum*. Nevertheless, recent evidence from Hill *et al.*[39] provides species-level support for the tumor association of *F. nucleatum*. They studied 898 tumor/paired-normal colorectal tissue samples using droplet digital polymerase chain reaction for *F. nucleatum* and found that normalized *F. nucleatum* counts were consistently higher in tumor tissue (0.02–0.06) than in matched normal tissue (0.001–0.006) across the epidemiologic risk factors examined. They also found that female sex was associated with both greater presence and enrichment of *F. nucleatum* in tumor tissue (odds ratio 1.58, 95% confidence interval 1.10–2.27; $p = 0.01$), whereas factors such as diet were not associated with *F. nucleatum* presence.[39] Previous studies have reported *F. nucleatum* promoting colorectal carcinogenesis through epithelial adhesion and invasion, activation of pro-inflammatory signaling pathways such as Toll-like receptor 4/nuclear factor-κB, and enhancement of a tumor-promoting microenvironment.[37,38,40,41] These findings together strengthen the interpretation that the recurrent Fusobacteria signal observed across our cohorts may reflect a biologically relevant association with CRC, while also emphasizing the need for species- and strain-level analyses to determine whether *F. nucleatum* specifically underlies the phylum-level pattern. The consistency of this signal across geographically and culturally distinct populations suggests that certain microbial drivers of CRC may be globally conserved.

In addition to conserved CRC-associated features, several lower-abundance phyla exhibited population-specific differences between CRC and control groups. Within the AA cohort, Spirochaetota and Cyanobacteria showed higher mean relative abundances in CRC compared with controls, increasing from 0.32% to 2.98% and from 0.64% to 1.40%, respectively. Similarly, Epsilonbacteraeota and Tenericutes were more abundant in WA_CRC samples than in controls, with mean relative abundances increasing from 0.59% to 3.29% and from 0.25% to 1.02%, respectively. The increase in Epsilonbacteraeota in WA_CRC is noteworthy because members of this phylum have previously been reported in association with CRC. For example, a recent study of mucosa-associated colonic microbiota in Indonesian patients reported higher abundances of *Campylobacter*, including *Campylobacter hominis*, in CRC compared with non-CRC controls.[42] However, because the present study assessed Epsilonbacteraeota only at the phylum level, it cannot determine which constituent genera or species contributed to the observed increase. These findings indicate that CRC-associated microbial differences extended beyond the dominant gut phyla but varied across populations, suggesting that less abundant microbial groups may contribute to cohort-specific patterns of dysbiosis.

4.1. Population-specific patterns of colorectal cancer-associated dysbiosis

Alpha diversity analyses revealed substantial regional differences in within-sample gut microbial diversity. Across both CRC and control cohorts, we observed significant population differences in Shannon diversity, Simpson diversity, and observed richness. Among CRC samples, SL_CRC samples generally exhibited lower diversity and observed richness than the U.S. and K cohorts, with significant differences observed between SL and AA and WA samples across multiple measures. K_CRC samples also exhibited significantly lower Shannon and Simpson diversity than WA samples and lower Simpson diversity than AA samples. These findings indicate that substantial population- and cohort-level differences in within-sample microbial diversity exist and that CRC-associated microbiome patterns should be interpreted relative to population-specific microbial baselines. Large multi-cohort CRC investigations by Wirbel *et al.*[43] and Thomas *et al.*[44] have similarly demonstrated that baseline microbial architecture varies substantially by cohort and geography, even when consistent CRC-associated signals are detectable across studies. Accordingly, the lower diversity observed among SL participants indicates that the observed diversity differences should be interpreted relative to region-specific ecological baselines rather than assuming a uniform diversity profile across populations.

Despite these population-level differences, statistical within-population comparisons did not identify significant differences in Alpha diversity measures between CRC and controls in any of the four populations. Although the distributions generally showed slightly higher alpha diversity among controls in the K and U.S. cohorts, and lower median diversity among SL_CRC samples, these descriptive differences were not statistically significant. These patterns are consistent with prior regional microbiome studies showing that geography and other population characteristics can influence CRC-associated microbial profiles rather than producing a uniform pattern across populations.[3,10,22,29] For example, regional studies such as Shi *et al.*[45] on Chinese cohorts reported that geography influences CRC-associated microbial composition. This supports our significant CRC-only population effect ($R^2 \approx 0.084$, $p = 0.001$) as biologically plausible and consistent with broader observations of population structuring. The increased variability observed in SL_CRC samples may reflect potential heterogeneity in dietary westernization, urbanization gradients, or healthcare access; factors that can amplify ecological instability under disease conditions.

Reports in the broader literature on alpha diversity remain consistent, with some studies observing reduced diversity in CRC and others reporting neutral or even increased richness depending on disease stage, sequencing depth, and analytical framework.[45,46] Hill *et al.*[39] reported lower Shannon diversity in colorectal tumor tissue than in patient-matched normal tissue in a large population-based study, demonstrating that reduced alpha diversity can occur in tumor-associated tissue in CRC. However, their analysis involved tumor and matched normal colorectal tissue from the same patients, whereas the present study compared CRC and control cohorts, using healthy controls in the SL and K cohorts and corresponding normal tissue in the AA and WA cohorts. These differences in study design and sample context may contribute to the differing alpha-diversity patterns observed across studies. Hill *et al.*[39] also found that dietary factors were not associated with tumor-tissue alpha diversity, further indicating that epidemiologic and dietary factors do not necessarily account for all variation in CRC-associated microbial diversity.

Similarly, Ma *et al.*[46] demonstrated that microbial diversity and community composition varied according to sample type, with differences observed among fecal, tumor, and paracancerous tissue microbiota. Their findings highlight the importance of considering the anatomical and biological source of microbiome samples when comparing diversity patterns across studies. In the present study, all samples were derived from colorectal tissue, reducing variation attributable to comparisons between fecal and tissue-associated microbiota; however, differences in population characteristics, clinical context, and cohort-specific methodology may still contribute to the observed regional variation.[26,34,39]

Beta diversity analyses have further demonstrated compositional restructuring in CRC. Previous multi-cohort studies have shown that CRC-associated microbial signatures can be detected across geographically distinct populations despite substantial background variation in community structure.[43,47] In the present study, the close overlap between AA and WA samples was consistent with the absence of a statistically significant difference between these U.S. CRC groups in PERMANOVA ($R^2 = 0.024$, $p = 0.85$). CRC-only PERMANOVA identified a significant association between population and microbial community composition across SL, K, and the U.S. ($R^2 = 0.077$, $p = 0.001$). This indicates that population background accounted for approximately 7.7% of the observed variation in microbial community structure. However, PERMDISP also identified significant differences in multivariate dispersion among CRC populations ($F = 5.37$, $p = 0.009$), suggesting unequal within-population variability across cohorts. Therefore, the significant PERMANOVA result should be interpreted with caution because differences in dispersion may contribute to the observed population effect. This interpretation is supported by PCoA, which revealed substantial overlap among CRC samples from all populations despite differences in dispersion. AA and WA samples clustered closely together, whereas K_CRC and SL_CRC samples exhibited broader distributions across the ordination space, indicating greater within-population heterogeneity rather than clear population-specific clustering. These findings are consistent with the broader literature demonstrating that CRC-associated microbial patterns coexist with substantial variation in community structure across populations, geographic regions, and sample types.[43,46,47] Ma *et al.*[46] similarly reported that beta-diversity patterns differed according to the biological source of the microbiome samples, highlighting the influence of methodological and cohort-specific factors on community structure. Importantly, because the present analysis was restricted to CRC samples, these results describe cross-population differences in microbial community composition among CRC cohorts and do not demonstrate population-specific CRC-associated dysbiosis. Establishing whether CRC-associated microbial alterations differ between populations would require direct evaluation of the CRC vs. control effect within each population and formal testing of population-by-disease interactions. Thus, the present findings indicate that while population background contributes to variation in CRC-associated microbiomes, much of the observed diversity

reflects substantial heterogeneity within populations. Therefore, population-associated differences in microbial community structure should be interpreted alongside differences in within-population dispersion and other sources of cohort variability.

4.2. Role of diet and lifestyle as potential drivers of population-specific microbiome and colorectal cancer risk differences

A central hypothesis of this study is that habitual dietary patterns, particularly the high-fat, low-fiber Western diet, contribute to population-specific differences in gut microbiome composition and CRC risk.[48,49] South Asian populations are undergoing a rapid nutritional transition characterized by a shift from traditional high-fiber, plant-based diets toward more Westernized dietary patterns enriched in red and processed meats, refined carbohydrates, and animal fats.[50-52] This contrasts with long-standing Westernized diets typical of U.S. populations and with traditional high-fiber dietary patterns historically observed in many African populations.[48,49] A recent study by Vásquez *et al.*,[53] in a systematic review of 16 studies, reported that Western dietary patterns were associated with alterations in gut microbial composition, intestinal inflammatory processes, and CRC-related outcomes, whereas anthocyanin-rich dietary interventions were associated with changes in microbial diversity and other potentially favorable biological effects. Osswald *et al.*[54] further provided experimental evidence linking Western diet exposure with CRC development through gut microbial secondary bile acid metabolism. In their experimental models, Western diet exacerbated CRC phenotypes and was accompanied by increased deoxycholic acid and microbial bile acid metabolism, while specific bile-acid-producing bacteria promoted tumor development in experimental systems. These findings provide biological context for the potential relationship among diet, the gut microbiome, and CRC, but they do not establish that dietary differences caused the population-associated microbiome patterns observed in the present study.

Recent epidemiological studies provide further evidence of an association between overall dietary patterns and CRC risk. Kase *et al.*[55] reported that greater adherence to a Dietary Index for Gut Microbiota, designed to characterize dietary quality in relation to gut microbial health, was associated with lower CRC risk during the first five years of follow-up in a large prospective cohort, although this association was not observed with longer follow-up. This finding provides epidemiological support for the possibility that dietary patterns capable of influencing the gut microbiome may also be relevant to CRC development. Similarly, Rosendal *et al.*[56] found that greater adherence to the EAT-Lancet dietary pattern, characterized by greater consumption of plant-based foods and lower consumption of red and processed meat, was associated with lower CRC risk in a prospective Danish cohort. Together, these studies suggest that overall dietary patterns may be relevant to CRC risk and may influence the gut microbial environment.

Consistent with this framework, AA_CRC and WA_CRC patients in this cohort showed broadly similar gut microbiome profiles at the phylum level, which may reflect shared dietary and lifestyle exposures. These similarities likely reflect common Westernized dietary patterns characterized by high intake of red and processed meats, reduced dietary fiber, and lower consumption of protective micronutrients such as beta-carotene, vitamin C and E, and calcium.[57,58] Previous meta-analyses have reported modest positive associations between red meat consumption and CRC risk. Even consumption of red meat ~50 g/day is associated with increased risk of colon cancer (risk ratio = 1.21, 95% confidence interval, 1.07–1.37).[57,59] For example, Farvid *et al.*[60] reported a pooled risk ratio of 1.10 (95% confidence interval, 1.03–1.17) for CRC when comparing the highest with the lowest categories of red meat intake. East-Asian studies, however, show less consistent associations (odds ratio 1.38, 95% confidence interval 0.98–1.93), suggesting that baseline dietary habits and consumption levels may modulate risk.[61] Several studies have shown that consuming lean meat reduces CRC risk compared with red meat consumption.[57,62] For instance, a study from Kerala, India, has shown a 20% decrease in CRC risk among populations consuming fish with every meal.[23] Another study in Fukuoka, Japan, has also demonstrated that high fish consumption can decrease CRC risk.[63] These findings highlight that South Asian populations undergoing Westernization may have varied risk profiles shaped by a mix of traditional and modern dietary exposures. CRC risk may be managed by replacing red and processed meat with lean meats, such as fish.[57,62]

While red meat provides essential amino acids that support microbial metabolic functions, it also contains heme iron, which has been associated with increased oxidative stress and mucosal proliferation. Consistent with this, epidemiological studies and large prospective cohorts, including the Nurses' Health Study and Health Professionals Follow-Up Study, have reported positive but heterogeneous associations between red and processed meat intake and CRC risk. The potential influence of diet on CRC may also extend beyond individual food components to the inflammatory potential of the overall diet. In a prospective cohort of Japanese men, Kotemori *et al.*[63] reported that greater dietary inflammatory potential, measured using the Energy-Adjusted Dietary Inflammatory Index, was associated with increased colon

cancer risk. The association also varied according to alcohol consumption, highlighting the importance of interactions between dietary patterns and other lifestyle factors.[9,36,64] These findings provide further support for considering diet within a broader lifestyle context.

4.3. Public health relevance

The rising incidence of CRC in LMICs parallels urbanization and the adoption of Western dietary patterns, with the gut microbiome functioning as a biological intermediary linking environmental change to carcinogenesis.[12,65,66] By showing that CRC-associated dysbiosis occurs within population-specific ecological baselines rather than as a globally uniform microbial state, this study provides a framework for understanding how dietary transition may reshape CRC risk in real time. This is particularly relevant for South Asian and African populations, where CRC incidence is increasing, but microbiome data remain comparatively limited.[27,49,50]

Diet and other lifestyle factors remain important public health considerations in CRC prevention. Independent evidence supports associations between dietary patterns, physical activity, body weight, alcohol consumption, and CRC risk, including evidence linking higher consumption of red and processed meat with increased CRC risk and higher consumption of whole grains and dietary fiber with lower CRC risk.[36,53] Therefore, population-level prevention efforts may benefit from promoting dietary patterns rich in whole grains, vegetables, fruits, and legumes while limiting red and processed meat and other established dietary and lifestyle risk factors.[36,53,57] Although the present study did not directly assess dietary intake, its findings highlight the potential relevance of dietary patterns as one modifiable factor contributing to population-specific microbiome profiles and CRC risk.

This study also addresses a significant knowledge gap. Most microbiome–CRC research has focused on Western/high-income populations, even though the steepest relative increases in CRC burden are occurring in transitional non-Western regions.[9,25,27,67] By incorporating SL and K cohorts alongside U.S. populations, this work facilitates a more globally representative understanding of microbiome–CRC relationships. Many LMICs face a "double burden" of persistent infectious diseases alongside rising non-communicable diseases, including CRC.[67,68] Limited screening programs, oncology infrastructure, and treatment access mean that rising CRC incidence often translates directly into higher mortality.[9,11] Preventive strategies targeting modifiable determinants such as diet and microbiome composition may therefore have a strong impact on these settings.

Beyond CRC, the microbiome offers a biological lens through which to understand the broader epidemiological transition occurring in urbanizing societies.[9,67] Public health strategies that preserve access to traditional diets, regulate the marketing of highly processed foods, and incorporate culturally appropriate nutrition policies would yield multidimensional health benefits. Finally, effective translation requires cultural sensitivity, local capacity building, and sustained global collaboration. Interventions in transitional populations must align with local dietary practices and sociocultural contexts to ensure acceptability and sustainability.[52,64,69] Continued international collaboration across diverse populations will be necessary to strengthen microbiome-based prevention strategies and ensure advances in microbiome science translate into globally applicable, context-sensitive public health solutions.

4.4. Limitations

Several limitations should be acknowledged when interpreting these findings. First, the source study was largely confounded with country/population; the available design does not permit population-associated biological effects to be analytically separated from source-study effects. Second, this study relied on publicly available datasets and associated metadata; therefore, it cannot be determined whether the observed microbial shifts preceded CRC development or result from tumor-associated ecological changes. Third, although combining cohorts improved overall representation, the studies included in this analysis have relatively modest sample sizes, which may limit statistical power to detect subtle population-specific effects or to fully characterize within-cohort heterogeneity.[27-29,70,71] This is particularly relevant when interpreting dispersion patterns or functional overlaps across geographically distinct groups. Fourth, methodological heterogeneity across cohorts may have influenced observed differences. Variability in sample collection, sequencing strategies, and bioinformatic approaches can affect taxonomic resolution and abundance estimates. Although standardized bioinformatic processing improved consistency across datasets, potential technical variations arising from the original study, laboratory, DNA extraction, polymerase chain reaction amplification, library preparation, and sequencing procedures could not be completely excluded. Additionally, reference databases and analytic tools are largely optimized for Western populations and may incompletely capture non-Western microbial diversity, potentially biasing taxonomic assignment or underrepresenting population-specific taxa.[22] Fifth, detailed dietary and lifestyle metadata were not available across the studied cohorts, limiting

the ability to model diet–microbiome–CRC mediation. Multiple additional factors, including host genetics, early-life exposures, antibiotic use, parasitic burden, hygiene, and environmental pollutants, may also greatly influence gut microbial composition.[65-67] These variables were not uniformly captured and may contribute to residual confounding. Publication bias toward positive associations between urbanization, microbiome shifts, and disease risk may further influence the apparent consistency of patterns across studies.

4.5. Future directions

Future studies should streamline their research by prioritizing functional, longitudinal, and multi-omics approaches to understand how changes in the gut microbiome relate to CRC over time. Studies focusing on transitional populations following non-Western diets would help determine how microbial dysbiosis arises with these diets and lifestyles. Mechanistic investigations, including controlled dietary interventions and experimental models, are needed to test whether specific taxa or metabolic pathways contribute directly to carcinogenesis. Expanded geographic coverage is also necessary. While Western cohorts are increasingly represented, data from South Asia, sub-Saharan Africa, Latin America, and other rapidly urbanizing LMIC regions remain limited.[66,72] Methodological refinement is needed, including functional pathway analysis using shotgun metagenomics, metabolomics, and integrated multi-omic frameworks to directly measure amino acid biosynthesis, bile acid metabolism, and one-carbon metabolic pathways.[34,73] Integrating microbial data with host inflammatory markers, transcriptomics, or epigenetic profiles would further strengthen our understanding of microbiome–host interactions in CRC. Finally, validating findings in independent cohorts and applying machine learning models across diverse populations would help determine whether these CRC-associated microbial signatures from transitional diets are broadly applicable or population-specific. Future studies incorporating direct dietary assessment alongside microbiome profiling may help determine whether dietary patterns, including higher fiber and plant-food intake and lower red and processed meat consumption, are associated with the microbiome differences observed across populations.

5. Conclusion

The findings from this study demonstrated that although Firmicutes and Bacteroidetes remained dominant across all groups, Fusobacteria showed a similar pattern of higher relative abundance in CRC compared with controls across the studied populations. This finding is consistent with previous reports identifying Fusobacteria as a CRC-associated microbial feature. However, because the present analysis was limited to phylum-level taxonomic resolution, the observed increase in Fusobacteria cannot be attributed to *F. nucleatum*. Alpha-diversity analyses showed no statistically significant differences between CRC and controls within any population for Shannon diversity, Simpson diversity, or observed richness. However, we observed significant population-level differences across both CRC and control cohorts for all three alpha-diversity measures. These differences may reflect diet, lifestyle, and environmental factors, although we did not directly measure them, and they remain potential explanations requiring further investigation. Beta diversity analysis indicated that CRC reduces clear population-level separation, yet population background still contributes meaningfully to microbial variability. In conclusion, this study highlights that while certain microbial signatures of CRC are conserved across populations, the extent and nature of microbiome alterations are strongly influenced by population-specific contexts. By comparing SL and K cohorts representing transitioning populations with AA and WA cohorts from the United States, this study underscores the importance of incorporating geographic and population diversity into CRC microbiome research. Future studies integrating detailed dietary, lifestyle, environmental, and clinical data, together with species-level and functional characterization of the microbiome, are needed to clarify the factors underlying these population-specific patterns and to determine whether the observed microbial features have potential clinical relevance.

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Conflict of interest

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Author contributions

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Ethics approval and consent to participate

Not Applicable

Consent for publication

Consent for publication was not applicable because this study used anonymized data, and no identifiable individual information is included in this manuscript.

Availability of data

The data supporting the findings of this study are available on GitHub at https://github.com/Vaishnavi-Chilakamarri12/Gut-Microbiome-Dysbiosis-and-CRC-in-LMICS

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