microorganisms

MDPI

Systematic Review

Gut Microbial and Associated Metabolite Markers for Colorectal Cancer Diagnosis

Areej A. Alhhazmi [1,*], Renad M. Alhamawi [1], Reema M. Almisned [2], Hanouf A. Almutairi [3], Ahdab A. Jan [4], Shahad M. Kurdi [1], Yahya A. Almutawif [1] and Waleed Mohammed-Saeid [5]

[1] Medical Laboratories Technology Department, College of Applied Medical Sciences, Taibah University, P.O. Box 344, Al-Madinah Al-Munawarah 42353, Saudi Arabia; rhamawi@taibahu.edu.sa (R.M.A.); msk8lab@gmail.com (S.M.K.); ymutawif@taibahu.edu.sa (Y.A.A.)
[2] Seha Polyclinic, P.O. Box 150, Al-Madinah Al-Munawarah 41311, Saudi Arabia; reemaalmisned@gmail.com
[3] Bioscience Program, Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), P.O. Box 6900, Thuwal 23955, Saudi Arabia; hanouf.almutairi@kaust.edu.sa
[4] Abdulla Fouad Medical Supplies and Services (AFMS), P.O. Box 150, Al-Madinah Al-Munawarah 21414, Saudi Arabia; ahdab.jan@abdulla-fouad.com
[5] Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, Taibah University, P.O. Box 344, Al-Madinah Al-Munawarah 42353, Saudi Arabia; wneyaz@taibahu.edu.sa
* Correspondence: ahazmib@taibahu.edu.sa

Abstract: Globally, colorectal cancer (CRC) is the second most common cause of mortality worldwide. Considerable evidence indicates that dysbiosis of the gut microbial community and its metabolite secretions play a fundamental role in advanced adenoma (ADA) and CRC development and progression. This study is a systematic review that aims to assess the clinical association between gut microbial markers and/or gut and circulating metabolites with ADA and CRC. Five electronic databases were searched by four independent reviewers. Only controlled trials that compared ADA and/or CRC with healthy control (HC) using either untargeted (16s rRNA gene or whole genome sequencing) or targeted (gene-based real-time PCR) identification methods for gut microbiome profile, or untargeted or targeted metabolite profiling approaches from the gut or serum/plasma, were eligible. Three independent reviewers evaluated the quality of the studies using the *Cochrane Handbook for Systematic Reviews of Interventions*. Twenty-four studies were eligible. We identified strong evidence of two microbial markers *Fusobacterium* and *Porphyromonas* for ADA vs. CRC, and nine microbial markers *Lachnospiraceae*-Lachnoclostridium, *Ruminococcaceae*-Ruminococcus, *Parvimonas* spp., *Parvimonas micra*, Enterobacteriaceae, *Fusobacterium* spp., Bacteroides, *Peptostreptococcus-Peptostreptococcus stomatis*, *Clostridia* spp.-*Clostridium hylemonae*, *Clostridium symbiosum*, and *Porphyromonas- Porphyromonas asaccharolytica* for CRC vs. HC. The remaining metabolite marker evidence between the various groups, including ADA vs. HC, ADA vs. HC, and CRC vs. HC, was not of sufficient quality to support additional findings. The identified gut microbial markers can be used in a panel for diagnosing ADA and/or CRC. Further research in the metabolite markers area is needed to evaluate the possibility to use in diagnostic or prognostic markers for colorectal cancer.

Keywords: gut microbiota; colorectal cancer; metabolites; 16s rRNA sequence; real-time PCR; CRC; ADA

Citation: Alhhazmi, A.A.; Alhamawi, R.M.; Almisned, R.M.; Almutairi, H.A.; Jan, A.A.; Kurdi, S.M.; Almutawif, Y.A.; Mohammed-Saeid, W. Gut Microbial and Associated Metabolite Markers for Colorectal Cancer Diagnosis. *Microorganisms* **2023**, *11*, 2037. https://doi.org/10.3390/microorganisms11082037

Academic Editor: Francesca Romana Ponziani

Received: 14 June 2023
Revised: 29 July 2023
Accepted: 30 July 2023
Published: 8 August 2023

1. Introduction

Globally, colorectal cancer (CRC) is the most frequently occurring cancer, ranking third in cancer incidence and second in mortality in 2020 and accounting for 1.9 million (10%) new cases and about 935,000 (9.4%) deaths around the world [1]. The rate of CRC incidence varies, with the highest reporting cases in Asia (52.3%) followed by Europe

(26.9%) and North America (9.3%). In 2020, there were about 4,007 (14.4%) new cases of CRC in Saudi Arabia, making it the most common cancer [2,3].

CRC is a heterogeneous disease that is usually defined as a carcinoma, mostly an adenocarcinoma (cancer of the glandular tissue) in the colon or rectum. It is formed when healthy cells in the lining of the colon or rectum commence to change and uncontrollably multiply, resulting in the formation of polyps or outgrowths [4].

The risk of developing CRC is influenced by many factors, especially environmental and genetic factors. Sex, age, and race are the most crucial elements to be considered in diagnosing CRC. Since colorectal cancer is an illness that is highly affected by gender, males are at a higher risk of developing colorectal cancer, which is approximately 44 percent higher than females [1]. Additionally, between 35 and 40 percent of colorectal cancer cases that are diagnosed have heritable causes, such as low-penetrance genetic mutations, hereditary cancer syndromes like Lynch syndrome, and other unidentified inherited genomic aberrations. With no family history or inherited genomic abnormalities, the remaining 60 to 65 percent of cases are random [1].

Microbiota is a complex microbial community that accounts for the integrity of their environment or the well-being of their hosts. The gastrointestinal tract is home to more than 10^{14} microorganisms, which includes almost ten times as many bacterial cells as human cells [5]. Microbiota contributes to many functions in the human body, such as immunological functions, metabolic functions, improving gut integrity, and shaping the intestinal epithelium. In the case of dysbiosis, the changes in microbial composition result in the disruption of these mechanisms [6]. Changes in the microbiota can lead to alteration in human inflammatory status and metabolites-generated by the host and gut-inhabited microbiota, which may directly or indirectly contribute to the etiology of CRC. The gut microbiota is recognized as an essential player in human illnesses such as obesity, inflammatory bowel disease, and colorectal cancer. Advancing facts suggest that microbial dysbiosis is strongly linked with the pathogenesis of intestinal tumors [7]. Recent metagenomics-based research has revealed that *Parvimonas micra*, *Solobacterium moorei*, *Fusobacterium nucleatum*, and *Peptostreptococcus stomatis* have enriched the gut of CRC patients [6]. Furthermore, an increased level of enterotoxigenic *Bacteroides fragilis* has been observed in the colonic mucosa and feces of CRC patients [8,9]. According to the bacterial driver-passenger model for CRC pathogenesis presented by Tjalsma et al. [10], CRC may be started by "driver" bacteria that are then replaced by "passenger" bacteria throughout carcinogenesis. However, it is still unclear how the human gut microbiota contributes to the development of CRC. Understanding the role played by the microbiome in the pathogenesis of CRC is crucial.

An early diagnosis of CRC raises the chances of survival and cure. CRC diagnosis relies largely on colonoscopy, which is an invasive procedure. In addition, performing CRC-specific antigens blood tests to identify carcinoembryonic antigen (CEA) and CA19-9, which are mainly used in the monitoring of CRC patients. One of the highly used tests for the diagnosis of CRC is stool-based tests, for example, gFOBTs which identify the presence of occult blood through the detection of heme pseudo peroxidase activity in the stool. However, the majority of these tests are expensive and exhibit low specificity and sensitivity [11]. Several studies have examined the composition of the gut's microbes to detect CRC biomarkers and relate certain pathogenic bacteria to CRC, such as *B. fragilis*, *F. nucleatum*, *Streptococcus bovis*, *E. coli*, *Enterococcus faecalis*, and *Porphyromonas* spp. [6]. Given the importance of gut microbiome profiling, which has been extensively conducted using 16S rRNA gene sequencing or shotgun metagenomics techniques [12], the direct link between the gut microbiota at the genus and the species levels, in addition to different CRC stages is challenging. Nevertheless, certain CRC microbial biomarker strains can be easily influenced by diet, antibiotics, hormone treatment, and chemotherapy.

In the case of CRC, disruption to the epithelial and mucous barriers, gastrointestinal inflammation, immunological escape, and genetic/epigenetic changes all work together to directly influence CRC development [8,13]. Numerous disorders, including type 1

diabetes, inflammatory bowel disease (IBD), and breast cancers, have been linked to metabolic changes [14–18]. Additionally, it has been shown that metabolites alter in the colon tissue, urine, serum, and feces of CRC patients as well as in CRC animal models [19–21]. Hence, accumulating numbers of metabolic markers have been proposed for CRC diagnosis, encompassing short-chain fatty acids [22], amino acids [23], bile acids (BAs) [24,25], tryptophan (Trp) metabolites [26], and L-carnitine metabolite (trimethylamine N-oxide) [27]. Additionally, few studies have linked gut bacteria dysbiosis to the altered metabolites in CRC.

This study aims to review relevant publications from five different databases to assemble gut microbial markers, gut metabolites, and circulating metabolites associated with CRC. Then, microbial biomarkers association with metabolites in CRC was collectively assessed. The analyzed data sets included those with stool or tissue microbiome sequencing, metabolomics profiling, and/or association studies examining the association between microbiome dysbiosis and CRC. The microbiome sequencing was either targeted for specific microbes using real-time PCR or untargeted, such as metagenomic sequencing or 16s rRNA gene sequencing. The metabolomics profiling for which targeted and untargeted based analyses using different hyphenated liquid chromatography—mass spectrometric (LC-MS) techniques of gut or plasma/serum samples were included.

2. Materials and Methods

In this systematic review of the literature, we used the *Cochrane Handbook for Systematic Reviews of Interventions* and examined the gut microbiota, gut metabolite indicators, and/or circulating metabolite markers as the intervention [28]. Our reporting was planned according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [29]. Literature search and study selection: a systematic search was conducted till 30 October 2022, using MEDLINE1, Google Scholar, Wiley, ScienceDirect, and Spring. Three experts (A.A.h, R.M.A, and W.M.S) in the fields of immunology, bioanalytical techniques, and microbiology collaborated to choose the search terms. The references cited in the listed publications were examined to find other studies. Five authors (Y.A.A, R.M.M, AAM, S.M.K, and A.A.J) selected studies that compared healthy controls with adenoma and/or carcinoma with respect to gut microbiome markers and/or gut and/or circulating metabolite markers, and their association for diagnosis or prognosis purposes. Following the selection, three authors (A.A.h, R.M.A, and W.M.S) reviewed the selected papers up until 30 December 2022; results from each database were reviewed, and duplicates were excluded (Figure 1).

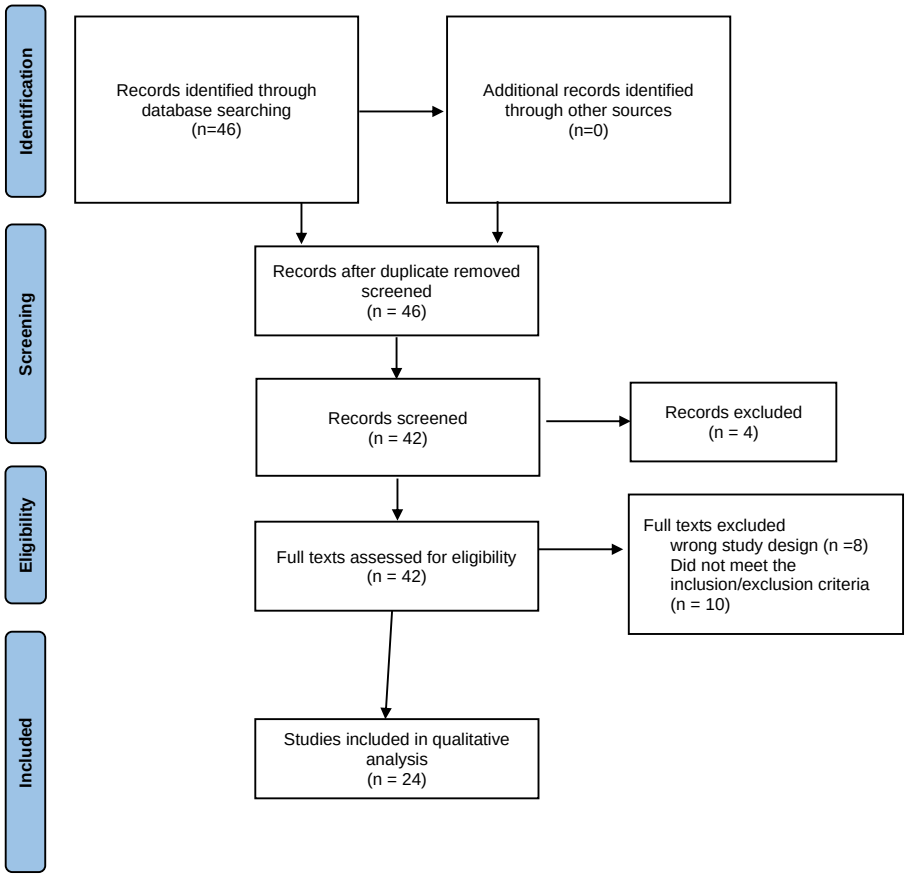

Figure 1. Search strategy guided by the PRISMA flow diagram [29].

The CRC group was defined as cancer patients where cancer starts in the colon or rectum. The development of CRC occurs in stages, starting with normal epithelium, progressing through a pre-malignant lesion (known as an adenoma), into a malignant lesion (carcinoma), which invades nearby tissues and has the potential to spread throughout the body (metastasis). The intervention was identified using the search term "colorectal cancer", "adenoma", "carcinoma", "polyps adenoma", and "sporadic carcinoma". The gut or intestinal microbiome was defined as the composition of microorganisms (bacteria, archaea, and eukaryota) colonizing the human gastrointestinal tract. Gut or intestinal microbiome intervention was identified using the search terms "gut or intestinal microbiota", "gut or intestinal microbiome", "gut or intestinal microbiome profile", "gut or intestinal microbiota profile", "gut or intestinal microbiome markers", and "gut or intestinal microbiota markers". Gut or intestinal and circulating metabolites were defined as small molecules that are generated as intermediate or end products of microbial metabolism in the gastrointestinal tract or intestinal and/or circulating system. The intervention was identified using the search term "gut or intestinal metabolites", "gut or intestinal metabolomic", "gut or intestinal metabolite profile", "gut or intestinal metabolomic profile", "gut or intestinal metabolite markers", "gut or intestinal metabolomic markers", "serum metabolites", "serum metabolomic", "serum metabolite profile", "serum metabolomic profile", "serum metabolite markers", "serum metabolomic markers", "plasma metabolites", "plasma metabolomic", "plasma metabolite profile", "plasma metabolomic profile", "plasma metabolite markers", "plasma metabolomic markers".

2.1. Eligibility Criteria

Only studies that compared healthy individuals to people diagnosed with adenoma or carcinoma and underwent peer review were considered. Reports on conference proceedings, case series with less than ten participants, case studies, systematic reviews, and protocol papers were all excluded. Three researchers (AAh, RA, and WMS) with a collective experience of more than ten years in the literature review chose the studies. The complete texts of the potentially suitable studies were retrieved after each title and abstract had been independently reviewed. At the titles and abstracts stage, disagreements were settled by consensus.

2.2. Data Extraction

Based on published guidelines, a standard form (Table S1) was created to retrieve data [30–32]. Three researchers (A.A.h, R.M.A, and W.M.S) extracted and cross-checked the data for each study. For each study, the following details were recorded: (1) Participant characteristics, including sample size, age, gender, and diagnosis; (2) Inclusion and Exclusion Criteria; and (3) Interventional features: untargeted; gut microbiome profile, untargeted gut/circulating metabolite profile, the association between gut microbiome species and colorectal cancer, the association between gut/circulating metabolite profile and colorectal cancer, and (4) characteristics of the outcomes: gut microbiome/genera/species, gut/circulating metabolites types.

Based on sensitivity, specificity, and area under the curve (AUC), the diagnostic performance of the investigated biomarkers was evaluated. If any of the data could not be directly described, the appropriate values were, if possible, calculated using other information.

2.3. Methodological Quality

The included studies' quality was evaluated in accordance with PRISMA and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [30]. The subject recruitment, examiners, methodology, results, handling of missing data, statistical analysis, and findings were the seven categories that were the focus of the quality review (Table S2). Each publication was critically analyzed independently by three reviewers (A.A.h, R.M.H, and W.M.S), and conclusions were confirmed by consensus. Prior to the thorough assessment, five full-text papers were evaluated and discussed for calibration. Studies were given a quality score based on a minimum threshold of 70%; those that met the threshold were deemed to be of good quality, and those that fell below it were assessed to be of low quality [31] (Table 1).

Table 1. Levels of evidence for summary statements and description of criteria adopted a priori to determine the level of evidence.

Level	Description
Strong	Consistent results (≥70%) from at least 2 high-quality studies
Moderate	1 high-quality study and consistent findings (≥70%) in 1 or more low-quality studies
Limited	Findings in 1 high-quality * study or consistent results (≥70%) among low-quality studies
NO	No study identified
Conflicting	Inconsistent results, irrespective of study quality

* Studies with quality scores over 70% were deemed high quality.

3. Results

3.1. Studies Included in the Review

After excluding duplicates, the search resulted in 42 references (Figure 1). A title and abstract screening resulted in the exclusion of 18 papers [32–49]. As a result, 24 papers in total met the criterion for selection. The most frequent reasons for exclusion were failing to meet the exclusion criteria (e.g., using animals in experiments or simply conducting bioinformatic analyses from databases) or using the incorrect study design (e.g., leaving out the healthy comparison group or CRC).

3.2. Comparison Groups/Subgroups of the Studies

Twelve studies included the three basic comparison groups; ADA, CRC, and HC, whereas ten studies included participants from CRC and HC only. Two studies had only two comparison groups, ADA and HC. All studies included both genders except one paper included only male participants, and in four studies, gender was not reported. Age range varied among the included studies, for which the youngest reported age was 18 yrs. Among the included studies, eight papers recorded cancer locations, and nine studies specified cancer stages (Table 2). Table 2 summarizes the study type, recruitment strategy, selection criteria, sample size, study frame time, and location.

Table 2. Description of study type and study participants in the included studies.

Author	Study Type	Recruitment Strategy and Selection Criteria	Number of Subjects and Groups	Location and Time Frame
Sun et al. [26]	Case-control study for untargeted microbiome and targeted metabolites identification, specifically Tryptophan and its metabolites in CRC patients	Male and female Aged 18-80 yrs ADA, CRC, HC	Healthy control = 38 ♂ 24 ♀ 14 56.85 yrs ± 10.99 ADA = 33 ♂ 23 ♀ 10 61.18 yrs ± 8.53 CRC = 46 ♂ 32 ♀ 14 63.63 yrs ± 11.39	The China–Japan Friendship Hospital, China March 2019 and December 2019
Kim et al. [50]	Case-control study for untargeted metabolites and microbiome identification in CRC patients Ps. The samples were obtained from cross sectional study, which gives this study a cross-sectional nature	All samples selected here have been enrolled in previous study [51] Male and female Aged 50–80 yrs ADA, CRC, and HC.	Healthy control = 102 ♂ 62 ♀ 40 50-59 yrs = 18 60-69 yrs = 49 >70 yrs = 35 ADA = 102 ♂ 62 ♀ 40 50-59 yrs = 17 60-69 yrs = 50 >70 yrs = 35 CRC = 6 ♂ 20 ♀ 16 50-59 yrs = 6 60-69 yrs = 19 >70 yrs = 11	ND 2001 to 2007
Nugent et al. [52]	Case-control study for targeted microbiota (Lactobacillus sp., Escherichia coli, Bifidobacterium sp., Clostridium sp., Bacteroides sp., and Eubacteria) and untargeted metabolites identification in CRC patients	Male and female Aged > 30 yrs ADA and HC	Healthy control = 15 ♂ 4 ♀ 11 55.0 yrs ± 1.1 ADA = 15 ♂ 6 ♀ 9 54.3 yrs ± 1.1	University of North Carolina Hospitals, USA ND
Chang et al. [53]	Case-control study for untargeted microbiome in CRC patients	Only Male Aged 38–77 yrs CRC and HC	Healthy control = 12 ♂ 12 CRC = 6 ♂ 6 Metagenomics sequences of 59 patients with CRC were obtained from the NCBI database (ref_CRC, Metagenomics sequencing data: PRJEB7774).	Haikou people's Hospital, Hainan, China ND
Guertin et al. [54]	Case-control study for targeted metabolites, trimethylamine N-oxide, Carnitine, Choline, and Betaine in CRC patients "Nested case-control study within the Alpha Tocopherol and Beta Carotene Cancer Prevention (ATBC) Study, described in detail elsewhere [55]	Gender ND Aged 50–69 yrs CRC and HC	Healthy control = 644 CRC = 644 **Tumor location** Proximal colon = 169 Distal colon = 153 Rectum ICD-9 = 282	USA ATBC study (1985–1988)–(1993) [55]
Kim et al. [56]	Case-control study for untargeted microbiome and untargeted metabolites in CRC patients	Male and female Aged 45–80 yrs CRC and HC	Healthy control = 40 ♂ 22 ♀ 18 49-78 yrs CRC = 32 ♂ 20 ♀ 16 45-80 yrs **Tumor Stage** 0 = 1 I = 7 II = 12 III = 9	CRC patients from Seoul National University Bundang Hospital and Chung-Ang University Hospital, South Korea HC individuals from Haewoondae Baek Hospital, South Korea April 2016–April 2018.

			IV = 3 **Tumor location** Cesum = 2 Ascending = 6 Transverse = 1 Sigmoid = 12 Rectal = 7	
Song et al. [57]	Pilot, case-control study for targeted metabolites, long and short fatty acid in CRC patients	Male and female Aged 45–70 yrs ADA, CRC, and HC	Healthy control = 28 ♂ 22 ♀ 6, 51.1 yrs ± 6.0 ADA = 27 ♂ 25 ♀ 1, 53.6 yrs ± 7.2 CRC = 26 ♂ 16 ♀ 10, 59.7 yrs ± 12.2 **Tumor stage** I = 3 IIa = 5 IIc = 1 IIIb = 11 IIIc = 3 IVa = 3 Presence of lymph node metastasis = 16 Presence of colonoscopic obstruction = 5 **Tumor location** Proximal cancer (above splenic flexure) = 3 Distal cancer (below splenic flexure) = 23	Asan Institute for Life Sciences, University of Ulsan College of Medicine, Korea July 2014 and August 2014
Genua et al. [58]	Case-control study for targeted metabolites, Acetic Acid, Propionic Acid, i-Butyric Acid, Butyric Acid, 2-MethylButyric Acid, i-Valeric Acid, Valeric Acid from serum in CRC patients	Male and female Cohort Irish and Czech Aged 45–70 yrs Tubular tubulovillous adenoma (TA/TVA), High-grade dysplasia (HGD), CRC, and HC	**Irish cohort 128** Healthy control = 36 ♂ 17 ♀ 19, 58 yrs ± 7 TA/TVA = 48 ♂ 30 ♀ 18, 61.5 yrs ± 11 HGD = 18 ♂ 11 ♀ 7, 59 yrs ± 7 CRC = 26 ♂ 13 ♀ 13, 56 yrs ± 23 **Czech cohort 85** Healthy control = 27 ♂ 12 ♀ 15, 56 yrs ± 10 CRC = 58 ♂ 40 ♀ 18, 64 yrs ± 15	The Adelaide & Meath Hospital in Dublin, Ireland Thomayer Hospital in Prague, Czech Republic.
D'asheesh et al. [59]	Case-control study for targeted microbiota Lactobaccilus acidophilus, Lactobacillus Plantarum, and Enterococcus faecalis	Aged 20–76 yrs Gender ND CRC and HC	Healthy control = 300 45.3± 2.5 CRC = 30055.34 ± 3.66	Iran March 2014 to October 2019
Coker et al. [60]	Case-control study for untargeted microbiome and targeted metabolites	Male and female Aged 58–83 yrs ADA, CRC, and HC	Healthy control 128 ♂ 59 ♀ 69, 64.03 yrs ± 6.84 ADA 140 ♂ 64 ♀ 54, 65.84 yrs ± 5.53 CRC 118 ♂ 64 ♀ 54, 73.21 yrs ± 10.37	Prince of Wales Hospital, the Chinese University of Hong Kong ND

Goedert et al. [61]	Case-control study for untargeted metabolites	Male and female Aged 46–75 yrs CRC and HC	Healthy control 102 ♂ 55.9% ♀ 44.1% 58.3 yrs ± 12.9 CRC 48 ♂ 64.6% ♀ 35.4% 62.9 yrs ± 13.7 **Tumor stage** Non-invasive = 20.8% Invasive, no known metastases = 41.7% Known metastases = 35.4% Missing = 2.1% **Tumor location** Right colon = 29.1% Left colon = 33.3% Rectal = 27.1% Missing = 10.4%	1985–1989 Washington DC area hospitals, USA
Sinha et al. [62]	Case-control study for untargeted microbiome and untargetd metabolites	Male and female Aged 45–76 yrs CRC and HC	Healthy control = 89 ♂ 55.5% ♀ 40.5% 58.4 yrs ± 13 CRC = 42 ♂ 59.5% ♀ 40.5% 63.4 yrs ± 13.1 **Tumor stage** Non-invasive = 21.4% Invasive, no known metastases = 42.9% Known metastases = 33.3% Missing = 2.1%	ND 1985–1987
Clos-Garcia et al. [63]	Case-control study for targeted metabolites as in [64] and untargeted microbiome identification in CRC patients	Male and female Aged >18 yrs ADA, CRC, and HC	Healthy control = 77 ♂ 35 ♀ 48 64.62 yrs ADA = 69 ♂ 41 ♀ 41 67.99 yrs CRC = 99 ♂ 60 ♀ 39 70.16 yrs	Samples batch 1 and 2 from COLONPREDICT study [65] Batch 3 from Instituto de Investigación Sanitario Galicia Sur, Spain ND
Tan et al. [66]	Case-control study for untargeted metabolites in CRC patients	CRC and HC Aged 24–82 yrs	Healthy control = 102 31-76 yrs CRC = 101 24-82 yrs **Tumor stage** I = 26 II = 43 III = 26 IV = 6 **Tumor location** Ascending = 21 Descending = 9 Sigmoid colon = 7 Rectum = 63	The Ruijin Hospital affiliated with Shanghai Jiao Tong University School of Medicine, China ND

Flemer et al. [67]	Case-control study for untargeted microbiome from stool and mucosa in CRC patients	Female and male Aged 27–82 yrs CRC, ADA, and HC	Healthy control = 56	Polyps ADA = 21	CRC = 59	Mercy University Hospital, Ireland ND
Zeller et al. [68]	Case-control study for untargeted microbiome from stool and mucosa in CRC patients	Female and male Aged 34–69 yrs Adenoma (small < 1 cm and large > 1 cm) HC from different cohorts from France and Germany	Healthy control = 358 Cohort France = 61 Cohort Germany = 297 **Cohort France = 61** **Tumor stage** 0 = 0 I = 15 II = 7 III = 10 IV = 21	ADA = 42 Cohort France ADA small = 27 ADA large = 15 **Cohort Germany = 38** **Tumor stage** 0 = 25 I = 0 II = 0 III = 13 IV = 0	CRC = 91	<u>F group</u> Assistance Publique - Hôpitaux de Paris (academic hospitals) <u>G population</u> the Department of Surgery at the University Hospital Heidelberg and the affiliated Hospital Salem <u>H population</u> From my microbe project http://my.microbes.eu/ ND
Zackular et al. [69]	Case-control study for untargeted microbiome from stool in CRC patients	Male and female Aged >18 yrs ADA, CRC, and HC	Healthy control = 30 ♂ 11 ♀ 19 55.3 yrs (±9.2)	ADA = 30 ♂ 18 ♀ 12 61.3 yrs (±11.1)	CRC = 30 ♂ 21 ♀ 9 59.4 yrs (±11)	Toronto (Canada), Boston (USA), Houston (USA), and Ann Arbor (USA) ND
Ohigashi et al. [22]	Case-control study for targeted metabolites and microbiome from stool in CRC patients	Male and female Aged 52–81 yrs ADA, CRC, and HC	Healthy control = 27 ♂ 16 ♀ 11 65.6 yrs ± 13.5	ADA = 22 ♂ 11 ♀ 11 66.6 yrs ± 9.2	CRC = 93 ♂ 49 ♀ 44 68.9 yrs ± 12.1	ND November 2007–October 2010
			Tumor stage Dukes A (36 patients) Dukes B (19 patients) Dukes C (24 patients) Dukes D (14 patients)			
Chen et al. [70]	Case-control study for untargeted metabolites and microbiome, followed by targeted microbiota using functional genes from stool in CRC patients	Male and female Aged 40–63 yrs ADA and HC	Healthy control = 30 ♂ 13 ♀ 17 50.33 yrs ± 10.87	ADA = 30 ♂ 20 ♀ 10 53.23 yrs ± 10.14		The First Affiliated Hospital of Kunming Medical University, China November 2017 to April 2018
Eklöf et al. [71]	Case-control study for targeted microbiome in CRC patients	Male and female Aged > 34 yrs CRC, ADA, HC	Healthy control = 65 ♂ 35 ♀ 30 34--80 yrs	Dysplasia ADA = 134 ♂ 80 ♀ 54 34-80 yrs	CRC = 39 ♂ 20 ♀ 19 34-80 yrs	The University Hospital in Umeå, Sweden September 2008 to March 2013
			Tumor stage I = 2 II = 21 III = 8 IV = 7			

			Tumor location		
				Total	Dysplasia	CRC
			Right	37	12	49
			Left	59	17	76
			Rectum	38	10	40
Gao et al. [72]	Case-control study for untargeted microbiome in CRC patients	Male and female Aged ND CRC, precancer (ADA), HC	Healthy control = 442 ♂ 60.65% ♀ 39.35% 65.79 yrs ± 12.73	Precancer (ADA) = 195 (31) ♂ 62.5% ♀ 37. 5% 63.07 yrs ± 12.84	CRC = 155 ♂ 29.48% ♀ 70.52% 64.96 yrs ± 10.44	The Shanghai Tenth People's Hospital, Tongji University School of Medicine and Changzheng Hospital affiliated with the Naval Medical University, China The discovery cohort from January 2014–November 2015 The validation cohort from March 2016–December 2017
			Tumor stage 0 = 25 (16.13%) I = 51 (32.9%) II = 56 (36.13%) III = 11.7 (10%) IV = 12 (7.74%)			
			Tumor location Ascending colon = 25 (16.13%) Transverse colon = 7 (4.52%) Descending colon = 10 (6.45%) Sigmoid colon = 33 (21.29%) Rectum = 70 (45.16%) Undefined = 5 (2.3%)			
Yusuf et al. [73]	Case-control study for targeted metabolites, short-chain fatty acids, acetate, propionate and butyrate acids in CRC patients	Male and female Aged >18 yrs CRC and HC	Healthy control = 14 ♂ 9 ♀ 5 50 yrs ±17.6		CRC = 14 ♂ 10 ♀ 4 53.8 yrs ±13.3	General Teaching Hospital Banda Aceh, Indonesia ND
Weir et al. [74]	Case-control study for untargeted microbiome and untargeted metabolites followed by targeted for short chain fatty acids in CRC patients	Male and female Aged >18 yrs CRC and HC	Healthy control = 11 ♂ 7 ♀ 3 50 yrs ±17.6		CRC = 10 ♂ 8 ♀ 2 53.8 yrs ±13.3	The University of Colorado Health-Poudre Valley Hospital in Fort Collins, CO, USA ND
			Tumor stage * T1 = 2 T2 = 3 T3 = 4 Tis = 1 * Tis: Carcinoma in situ: intraepithelial or invasion of lamina propria; T1: Tumor invades submucosa; T2: Tumor invades muscularis propria; T3:Tumor invades through			

			muscularis propria into the subserosa or into nonperitonealized pericolic or perirectal tissue. **Tumor location** Ascending 3 Rectum 3 Sigmoid 4		
Yang et al. [75]	Case-control study for untargeted microbiome and metabolites in CRC patients	Male and female Aged >60 and <60 yrs CRC and HC	Healthy control = 50 ♂ 17 ♀ 33 >60 yrs = 33 <60 yrs = 17	CRC = 50 ♂ 26 ♀ 24 >60 yrs = 24 <60 yrs = 26	Ongji University Affiliated Tenth People's Hospital (Shanghai, China) January 2014 to September 2014

3.3. Interventions of the Included Studies

Of the 24 studies meeting the inclusion criteria, 11 papers investigated both gut microbiome and associated metabolites, seven papers profiled only gut microbiome, and six described associated metabolites in CRC patients. Thirteen studies conducted an untargeted gut microbiome technique, whereas four performed targeted methods among the included studies. One study performed untargeted microbiome profiling, followed by the targeted method. For metabolites profiling, eight studies employed an untargeted profiling technique, and one study did the untargeted followed by the targeted method. Eight studies used the targeted metabolite method (Table 3).

Table 3. Description of the intervention used in the included studies.

Author	Group	Intervention		Sample Type	Metric
		Targeted metabolites identification	Untargeted microbiome identification		
Sun et al. [26]	Experimental group AD and CRC Control group	Tryptophan (Trap) and its metabolites, such as L-Trp, L-Kynurenine (KYN), indole, skatole, indole-3-carboxylic acid (I3CA), Indole-3-aldehyde (IALD), Indole-3-acetate (IAA), Indolepropionic acid (IPA), indoxyl-3- sulfate (I3S), and Indole-3-acetadehyde (IAALD) using Ultraperformance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) analysis	16S geneRNA gene sequencing using an Illumina NovaSeq PE250	Fecal specimen	+/− of Trp and its metabolites Indole/Trap ratio Distribution (abundance) at bacterial genera level
Kim et al. [50]	Experimental group AD and CRC Control group	Untargeted metabolites identification	Untargeted microbiome identification	Fecal specimen	Distribution (abundances) of metabolites Distribution (abundance) bacterial genera
		UPLC- MS/MS platform	16S gene RNA gene sequencing using the Illumina MiSeq system		
Nugent et al. [52]	Experimental group AD Control group	Untargeted metabolites identification	Targeted microbiome identification	Rectal mucosal biopsy	+/− of metabolites Distribution (abundance) of bacterial genera/species
		Liquid chromatography and gas chromatography time of flight mass spectrometry	For *Lactobacillus* sp., *Escherichia coli*, *Bifidobacterium* sp., *Clostridium* sp., *Bacteroides* sp., and *Eubacteria* using qPCR with primers that amplify 16S rDNA		
Chang et al. [53]	Experimental group CRC Control group	Untargeted microbiome identification		Fecal specimen	Distribution (abundance) of bacterial species
		Whole-genome shotgun sequencing Illumina HiSeq			
Guertin et al. [54]	Experimental group	Targeted metabolites identification		Serum specimen	

	CRC Control group	Trimethylamine N-oxide, Carnitine, Choline, and Betaine in CRC patients using liquid chromatography (LC) tend mass spectrometry (MS)			+/− of serum metabolites, trimethylamine N-oxide, Carnitine, Choline, and Betaine Odds ratio of serum metabolites, trimethylamine N-oxide, Carnitine, Choline, and Betaine
Kim et al. [56]	Experimental group CRC Control group	Untargeted metabolites identification Gas chromatography-time-of-flight mass spectrometry	Untargeted microbiome identification 16S gene RNA gene sequencing by MiSeq Illumina.	Stool to extract bacterial extra vesicles (EV)	Distribution (Abundance) of metabolites Fold change difference of the means Distribution of bacterial genera
Song et al. [57]	Experimental group CRC Control group	Targeted metabolites identification Long and short fatty acids using gas chromatography—mass spectrometry		Fecal specimen	Distribution (Abundance) of metabolites Mean ± SD
Genua et al. [58]	Experimental group TA/TVA HGD CRC Control group	Targeted metabolites identification Acetic Acid, Propionic Acid, i-Butyric Acid, Butyric Acid, 2-MethylButyric Acid, i-Valeric Acid, Valeric Acid using gas chromatography		Plasma specimen	+/− of the following metabolites, Acetic Acid, Propionic Acid, i-Butyric Acid, Butyric Acid, 2-MethylButyric Acid, i-Valeric Acid, Valeric Acid Mean/IQ
D'asheesh et al. [59]	Experimental group CRC Control group	Targeted microbiome identification *Lactobacillus acidophilus*, *Lactobacillus palntarom and Enterococcus faecalis* By real-time PCR		Fecal specimen	Fold change and CFU/ml
Coker et al. [60]	Experimental group ADA and CRC Control group	Targeted metabolites identification Methyl and ethyl chloroformate (MCF and ECF) derivatized compounds identified previously using gas chromatography coupled to time-of-flight mass spectrometer (GC-TOFMS) analysis	Untargeted microbiome identification Whole-genome shotgun sequencing of all samples was carried out on an Illumina HiSeq.	Fecal specimen	Distribution (Abundance) of metabolites Fold change Distribution (Abundance) of bacterial species
Goedert et al. [61]	Experimental group	Untargeted metabolites identification		Fecal specimen	

	CRC Control group	High-performance liquid chromatography/ tandem mass spectrometry			Distribution (Abundance) of metabolites
Sinha et al. [62]	Experimental group CRC Control group	Untargeted metabolites identification HPLC-GC/MS-MS	Untargeted microbiome identification 16S rRNA gene sequencing	Fecal specimen	Distribution (Abundance) of metabolites Distribution of bacterial genera Odds ratio for both microbiota and metabolites
Clos-Garcia et al. [63]	Experimental group ADA, CRC Control group	Targeted metabolites identification UHPLC-MS	Untargeted microbiome identification 16S rRNA gene sequencing	Fecal specimen	Distribution (Abundance) of metabolites Distribution of bacterial genera
Tan et al. [66]	Experimental group CRC Control group	Untargeted metabolites identification Gas chromatography time-of-flight mass spectrometry (GC−TOFMS)UPLC−QTOFMS		Serum specimen	Distribution (Abundance) of metabolites %
Flemer et al. [67]	Experimental group ADA CRC Control group	Untargeted microbiome identification 16S rRNA gene sequencing		Fecal specimen and mucosa biopsy	Distribution of bacterial species
Zeller et al. [68]	Experimental group ADA CRC Control group	Untargeted microbiome identification Whole-genome shotgun sequencing of fecal samples) 16S rRNA gene sequencing (DNA from 48 tissue sample pairs (tumor and healthy mucosa) and 129 fecal samples		Fecal specimen and mucosa biopsy	Distribution (Abundance) of bacterial genera
Zackular et al. [69]	Experimental group ADA CRC Control group	Untargeted microbiome identification 16S rRNA gene sequencing analysis		Fecal specimen	Distribution (Abundance) of bacterial genera
Ohigashi et al. [22]	Experimental group ADA CRC Control group	Targeted metabolites identification Organic acids, identification from stools using high-performance liquid chromatography system.	Targeted microbiome identification *Clostridium leptum*, *Bacteroides fragilis*, *Bifidobacterium*, *Atopobium*, *Prevotella*, *Clostridium difficile*, *Clostridium perfringens*, *Lactobacillus casei*, *Lactobacillus gasseri*,	Fecal specimen	Distribution (Abundance) of metabolite. Bacterial counts

			Lactobacillus plantarum, *Lactobacillus reuteri*, *Lactobacillus ruminis*, *Lactobacillus sakei*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus fructiborans Enterobacteriaceae*, *Enterococcus*, *Staphylococcus*, *Pseudomonas* using real-time PCR		
Chen et al. [70]	Experimental group ADA Control group	Untargeted metabolites identification	Untargeted microbiome identification	Fecal specimen	Abundance/ distribution and concentration of metabolite. Bacterial species distribution/ abundance Fold-change in gene expression of bacterial species producing specific metabolites.
		Ion chromatography and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).	16S rRNA gene sequencing analysis followed by real-time PCR to identify bacteria that produced specific metabolites		
			Targeted microbiome identification		
			Real-time PCR analysis, butyrate-producing bacteria, determined by the presence of the butyryl-coenzyme-A-CoA transferase (*bcoA*) gene, secondary bile acid-producing bacteria, determined by the presence of the Bile acid 7α-dehydroxylation (*baiCD*) gene, conjugated linoleic acid-producing bacteria, determined by the presence of the plasminogen activator inhibitor 1(*pai-1*) gene, plasmid-encoded *cfr* gene (*clbA*) gene and the polypeptide outer membrane usher protein (*afa*C) gene of the *afa-1* operon were used to detect Putative inactive phenolphthiocerol synthesis polyketide synthase type I (*pks1*) bacteria and afa-1 adhesin-expressing diffusely adhering *Escherichia coli*		

			(DAEC), respectively For *F. nucleatum* 16S rRNA gene		
Eklöf et al. [71]	Experimental group ADA/dysplasia CRC Control group	Targeted microbiome identification		Fecal specimen	+/− of *clbA* and *afaC* +, *F. nucleatum* bacteria
		qPCR *clbA* gene colibactin-producing bacteria, diffusely adherent *Escherichia coli* harboring the afa-1 operon, and *F. nucleatum*			
Gao et al. [72]	Experimental group ADA CRC Control group	Untargeted microbiome identification		Fecal specimen	Distribution (Abundance) of bacterial species
		16S rRNA gene sequencing analysis			
Yusuf et al. [73]	Experimental group CRC Control group	Targeted metabolites identification		Fecal specimen	+/− absence of acetate, propionate and butyrate acids
		Acetate, propionate and butyrate acids by gas chromatography			
Weir et al. [74]	Experimental group CRC Control group	Untargeted metabolites identification	Untargeted microbiome identification	Fecal specimen	Distribution (Abundance) of bacterial species, % abundant, fold change Distribution (abundance)
		Gas chromatography-mass spectrometry (GC-MS)	16S rRNA gene sequencing analysis		
		Targeted metabolites identification			
		Gas chromatography-mass spectrometry (GC-MS)			
Yang et al. [75]	Experimental group CRC Control group	Untargeted metabolites identification	Untargeted microbiome identification	Fecal specimen	Distribution (Abundance) of bacterial species,
		Gas chromatography-mass spectrometry (GC-MS)	16S rRNA gene sequencing analysis		

The majority of the studies (9 out of 11) conducted both microbiome and metabolite profiling using fecal specimens. One study used rectal mucosa biopsy, and another study used stool to extract bacterial extra vesicles (EV). All but one of the seven studies that only focused on microbiome profiling used fecal specimens. The remaining study used rectal mucosa biopsy along with the fecal specimen. For metabolite profiling studies, three studies used fecal specimens, two used serum specimens, and one used plasma specimens. From the resulting 24 studies, we reported the outcome measurement of metabolites as (distribution of metabolite types) (Table 3). Microbiome outcomes were documented as (the distribution of different genera/species in the different study groups and fold change of specific gene expression of particular species). Table 3 summarizes the interventions, the comparison groups, the specimen type, and the metric used in the included studies.

Five studies (Flemer et al. [67], Zeller et al. [68], Zacular et al. [69], Eklöf et al. [71], and Gao et al. [72]) investigated only bacteria as biomarkers and also reported AUCs for diagnostic evaluation. According to Zeller et al. [68], six bacteria differentiated between CRC and healthy controls with an AUC of 85% (84–87%); similarly, Flemer et al. [67] identified six bacteria that distinguished between CRC and healthy controls with an AUC of 87%. Eklöf et al. [71] showed that only one bacterium can differentiate between ADA and CRC with an AUC of 73.1%, yet with 84.6% sensitivity and 63% specificity. Six, four, and six bacteria were used to identify ADA vs. HC, ADA vs. CRC, and CRC vs. HC with AUC values of 79.8% (687–90.8%), 82.3% (72.2–92.3%), and 83.9% (74–93.8%), respectively, as reported by Zacular et al. [69]. Gao et al. [72] showed AUCs of 61.6% (52–71%) (sensitivity: 83.6% and specificity: 39%) and 85.8% (78–93%) (sensitivity: 66.7% and specificity: 98%) for when 18 bacterial species implemented for the diagnosis of ADA or CRC, respectively (Table 4).

Table 4. Included studies identified microbial and metabolites associated with ADA or CRC for diagnostic purposes.

Author	Comparison Group	Bacterial or Metabolite Markers	Performance to Detect ADA or CRC		Identification Technique
			AUC (CI 95%)	Sen/Spec	
Sun et al. [26]	ADA vs. HC	3 metabolites IPA IALD Indole/Trap ratio	ND	ND	16S rRNA gene sequencing. Ultraperformance liquid chromatography coupled to tandem mass spectrometry.
	ADA vs. HC	4 metabolites Skatole IALD I3CA Indoles	ND	ND	
	CRC vs. HC	10 Bacteria *Bacteroides* *Bacilli* Clostidales_Incertae_Sedis XI Clostridia Fusobacteria Verrucomicrobia Corynebacteriacea Enterobacteriacea 5 metabolites KYN IPA IALD I3CA Indole/Trap ratio	ND	ND	
Kim et al. [50]	AD vs HC	24 metabolites Endocannabinoid	ND	ND	UPLC-MS/MS platform

	N acetyl-cadverine		
	Bilirubin ZZ		
	Lionleoyl ethanolamide		
	Oleoyl ethanolamide		
	Palmitoyl ethanolamide		
	3-Hydroxy-palmitate		
	Myristoleate		
	Palmitoleate		
	1-Linoleoyl-GPE		
	1-Palmitioyl -GPE		
	Secondary bile acid		
	3b-Hydroxy-5-cholenoic acid		
	Deoxycholate		
	Polyunsaturated fatty acid		
	Docosahexaenoate		
	Docosapentaenoate		
	Hexadecadienoate		
	Sphingolipid		
	N-palmitoyl-saphinganine		
	Hexadecasphinganine		
	Sphinganine		
	Piperine		
	3,7-Dimethyl-urate		
CRC vs. HC	8 metabolites Polyunsaturated fatty acid Docosahexaenoate Docosapentaenoate Hexadecadienoate Sphingolipid N-palmitoyl-saphinganine Hexadecasphinganine Sphinganine Piperine	ND	ND

		3,7-Dimethyl-urate			
Nugent et al. [52]	ADA vs. HC	23 metabolites Galactose, 13,14-dihydro-15-keto-PGE2, 5-oxoproline, 2,4-diaminobutyric acid, Pentadecanoic acid, 5-hydroxyindoleacetic acid, Phosphoric acid, 2-aminoethanol, Dihydroceramide, Ornithine, linoleic acid, Petroselinic acid, LysoPC (18:2(9Z,12Z)), Myo-inositol, Diketogulonic acid, Prostaglandin E2, Methionine, 2-aminobutyric acid, Oleamide, Glycine, Maltitol, 2-phenylglycine, 2-phenylacetamide, N6-acetyl-L-lysine	ND	ND	Liquid chromatography and gas chromatography time of flight mass spectrometry
Chang et al. [53]	CRC vs. HC	18 bacteria *Parvimonas micra* *Fusobacterium nucleatum* *Clostridium saccharoperbutylacetonicum* *Clostridium beijerinckii* *Eubacterium celluloslvens* *Lachnoclostridium phytofermentans* *Clostridium butyricum* *Herbiirix luporum* *Balcillus cereus* *Blautia sp. SCOSB48* *Anaerobutyrucium hallii* *Lachnospiraceae bacterium Choco86* *Eubacterium eligens* *Blautia hansenii* *Longibaculum SPKGMB06250* *Clostridum sporogenes* *Faecalibacterium prausnitizi* *Anaerostipes hardus*	ND	ND	Whole-genome shotgun sequencing
Guertin et al. [54]	CRC vs. HC	1 metabolite Serum choline	ND	ND	Liquid chromatography (LC) tandem mass spectrometry (MS)
Kim et al. [56]	CRC vs. HC	2 Bacteria *Solanum melongena, Collinsella*	95%	ND	16S rRNA gene sequencing Gas chromatography-time-of-flight mass spectrometry
		2 metabolites Leucine and Oxalic acid	92%	ND	

		Both bacteria+ metabolites *Solanum melongena*, *Collinsella*, Leucine and Oxalic acid	100%	ND	
Song et al. [57]	CRC vs. HC	4 metabolites Monounsaturated fatty acid (MUFAs), Oleic acid, ω-6-polyunsaturated fatty acids (ω-6 PUFAs), and Linoleic acid	ND	ND	Gas chromatography-mass Spectrometry
Genua et al. [58]	ADA vs. CRC	1 metabolite 2-MethylButyric acid			Gas chromatography
	CRC vs. HC	4 metabolites Acetic acid, Propnic acid, i-Valeric, and Valeric acid	ND	ND	
D'asheesh et al. [59]	CRC vs. HC	3 Bacteria *Lactobacillus acidophilus*, *Lactobacillus palntarom, and Enterococcus faecalis*	ND	ND	Real-time PCR
Coker et al. [60]	ADA vs. CRC	6 bacteria *Roseburia inulinivorans* *Xanthmonas perforans* *Fusobacterium nucleatum* *Eiknella corrodens* *Parvimonas micra* *Peptostreptococcus anaerobius* 11 metabolites 2-Hydroxy butyric acid Gamma Aminobutyric acid L-alanine L-Aspartic acid Norvaline Orinthine Oxoadipic acid Oxoglutaric acid Palmitoleic acid Pimelic acid	Only bacteria 94.17% (91.5–96.83)	ND	Whole-genome shotgun sequencing Gas chromatography coupled to time-of-flight mass Spectrometer (GC-TOFMS)
	ADA vs. HC	14 bacteria *Roseburia inulinivorans*	Only bacteria	ND	

		Xanthmonas gardneri *Fusobacterium nucleatum* *Prevotella intermedia* *Peptostreptococcus stomatis* *Sutterella parviruba* 4 metabolites Alpha-Linoleici acid L-Homoserine Phenylacetic acid Phenyllactic ac	87.59% (83.58, 91.6%)		
	CRC vs. HC	14 bacteria *Eubacteria cellulosolvens* *Lachinospiraceae_bacterium-3-1-57FAA-CT1* *Clostridium bolteae* *Streptococcus tigurinus* *Xanthmonas gardneri* *Eikenella corrodens* *Oscillibacter valericigens* *Actinomyces viscosus* *Synergistes_sp_1_syn1* *Clostridium symbiosum* *Prevotella intermedia* *Slackia exigua* *Prevotella nigrescens* *Porphymonas gingivalis* 2 metabolites L-Asparagine Phenyllactic acid	Both 14 bacteria and 2 metabolites 93.7% (91.07, 96.42%)	ND	
Goedert et al. [61]	CRC vs. HC	10 metabolites 3-Dehydrocarnitine, p aminobenzoate (PABA) α-Tocopherol, γ-Tocopherol, Pterin, N-2-Furoyl-glycine, p-Hydroxybenzaldehyde, Sitostanol, Conjugated linoleate-18-2N7, Palmitoyl-sphingomyelin, Mandelate	77%	ND	High-performance liquid chromatography/tandem mass spectrometry

Sinha et al. [62]	CRC vs. HC	4 Bacteria *Fusobacterium*, *g- Porphyromonas*, Clostridia, Lachnospiraceae 5 metabolites p-hydroxy-benzaldehyde, Palmitoyl-sphin-gomyelin p-aminobenzoate, Conjugated linoleate, and Mandelate	ND	ND	16S rRNA gene sequencing HPLC-GC/MS-MS
Clos-Garcia et al. [63]	ADA vs. H	1 metabolite Triacylglycerol	ND	ND	16S rRNA gene sequencing UHPLC-MS
	ADA vs. CRC	4 Bacteria *Streptococcus* *Parvvimonas* *Coriobacteriaceae* *Adlercreutzia* 3 metabolites cholesteryl esters, sphingolipids, Glycerophospatidylcholine	ND	ND	
	CRC vs. HC	7 Bacteria *Fusobacterium*, *Streptococcus*, *Parvimonas*, *Coprococcus*, *Blatia*, *Clostridum*, *Staphylococcus* 3 metabolites Cholesteryl esters, sphingolipids, Glycerophospatidylcholine	ND	ND	
Tan et al. [66]	CRC vs. HC	72 metabolites This involved the following categories: Tricarboxylic acid (TCA) cycle, urea cycle, glutamine, fatty acids, and gut flora metabolism Tan et al. [66]	ND	ND	Gas chromatography time-of-flight mass spectrometry (GC−TOFMS) UPLC−QTOFMS
Flemer et al. [67]	CRC vs. HC	6 Bacteria *Bacteroides* *Roseburia* *Ruminococcus* *Oscillibacter* *Lachinospiraceae incertae* *Coporoccus*	87%	ND	16S rRNA gene sequencing
Zeller et al. [68]	CRC vs. HC	2 Bacteria *Fusobacterium nucleatum* subsp. vincentii and *Fusobacterium nucleatum* subsp animalis	85% (84–87%)	ND	Whole-genome shotgun sequencing /16S rRNA gene sequencing

Zackular et al. [69]	ADA vs. HC	6 Bacteria *Fusobacterium*, *Porphyromonas*, *Lachnospiraceae*, Enterobacteriaceae, *Bacteroides*, Lachnospiraceae Clostridiales	79.8% (68.7–90.8%)	ND	16S rRNA gene sequencing
	ADA vs. CRC	4 Bacteria *Fusobacterium*, *Porphyromonas*, *Parasutterella* *Pacscolarctobacterium*	82.3% (72.2–92.3%)	ND	
	CRC vs. HC	6 Bacteria *Fusobacterium*, *Porphyromonas*, *Lachnospiraceae*, Enterobacteriaceae, *Bacteroides*, Lachnospiraceae and Clostridiales	83.9% (74–93.8%)	ND	
Ohigashi et al. [22]	ADA vs. CRC	3 Bacteria *Clostridium leptum*, *Bacteroides fragilis*, *Staphylococc*	ND	ND	Real-time PCR Liquid chromatography system
	CRC vs. HC	7 Bacteria *C. coccoides*, *C. leptum*, *B. fragilis*, *Bifidobacterium*, *Atopobium*, Enterobacteriaceae, *Staphylococcu* 4 Metabolites Acetic acid, Propionic acid, Butyric acid, and Valeric acid	ND	ND	
Chen et al. [70]	ADA vs. HC	1 Bacterium *Bacteroidete* 3 Metabolites Acetic acid, butyric acid, and *t10*, *c12-CLA*	Both 90% (70–90%)	ND	16S rRNA gene sequencing analysis followed by real-time PCR. Ion chromatography and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).
Eklöf et al. [71]	ADA/dysplasia vs. CRC	1 Bacterium *F. nucleatum*	73.7%	84.6% and 63.1%	Real-time PCR
Gao et al. [72]	ADA vs. HC and CRC vs. HC	18 Bacteria *Rhodococcus*, *Anaerostipes*, *Escherichia_Shigella*, *Akkermansia*, *Gemella*, *Clostridium_XVIII*,	ADA vs. HC 61.6% (52–71%)	ADA vs. HC 83.6% and 39%	16S rRNA gene sequencing

		Alkaliphilus Paenibacillus, *Enterococcus*, *Fusobacterium*, *Fusicatenibacter*, *Blautia Porphyromonas*, *Faecalibacterium*, *Parvimonas*, *Peptostreptococcus*, *Clostridium_IV Bacillus*	CRC vs. HC 85.8% (78–93%)	CRC vs. HC 66.7% and 98%	
Yusuf et al. [73]	CRC vs. HC	3 Metabolites Acetate, propionate and butyrate acids	ND	ND	Gas Chromatography
Weir et al. [74]	CRC vs. HC	18 Bacteria *Bacteroides finegoldii*, *Bacteroides intestinalis*, *Prevotella copri*, *Prevotella oris*, *Ruminococcus obeum*, *Dorea formicigenerans*, *Lachnobacterium bovis*, *Lachnospira pectinoschiza*, *Pseudobutyrivibrio ruminis*, *Bacteroides capillosus*, *Ruminococcus albus*, *Dialister invisus*, *Dialister pneumosintes*, *Megamonas hypermegale*, *Acidaminobacter unclassified*, *Phascolarctobacterium unclassified*, *Citrobacter farmer*, *Akkermansia muciniphila*,	ND	ND	16S rRNA gene sequencing analysis Gas chromatography—mass spectrometry (GC-MS)
		20 Metabolites Alanine, Glutamate, Glycine, Aspartic acid, Leucine, Lysine, Proline, Threonine, valine, Phenylalanine, Benzeneacetic acid, Propionic acid, pantothenic acid, Cholesterol derivatives, Oleic acid, Linoleic acid, Elaidic acid, Glycerol, Monooleoylglycerol, Ursodeoxycholic acid	ND	ND	
Yang et al. [75]	CRC vs. HC	13 Bacteria *Escherichia-Shigella*, *Parvimonas*, *Fusobacterium*, *CFT112H7_norank*, *Porphyromonas*. *Firmicutes*, *Clostridiales*, *Clostridia*, *Lachnospiraceae*, *Ruminococcaceae*, *Selenomonadales*, *Negativicutes*, and *Faecalibacterium*	ND	ND	Gas chromatography—mass spectrometry (GC-MS) 16S rRNA gene sequencing analysis
		2 metabolites Cadaverine putrescine	Only metabolites, each one alone: 74% 67.2	ND	

Two studies (Yang et al. [75] and Godert et al. [61]) reported only metabolites as bio-indicators and evaluated CRC diagnostic implementation. According to Yang et al. [75], two metabolites, cadaverine and putrescine, can be used to identify CRC with AUCs of 77% and 67.2, respectively. An AUC of 77% based on 10 metabolites was reported by Godert et al. [61] (Table 4).

Three studies (Kim et al. [56], Coker et al. [60], and Chen et al. [70]) evaluated the diagnostic application of both biomarkers, bacteria, and metabolites. According to Kim et al. [56], using the identified bacteria alone can have an AUC of 95%, and the two metabolites alone can generate an AUC of 92%; however, combining the two bacteria and the two metabolites improved the AUC to 100%. An AUC of 94.7% (91.5–96.83%) and 87.59% (83.58–91.6%) based on only 6 bacteria and 14 bacteria differentiated between ADA vs. CRC and ADA vs. HC, respectively. However, when the 14 bacteria were combined with the two metabolites, the AUC was 93% (91.07–96.42%) for CRC diagnosis by Coker et al. study [60]. When *Bacteroidetes* was combined with Acetic acid, butyric acid, and *t10, c12-CLA*, they exhibited an AUC of 90% (70–90%) to differentiate prelesion (ADA) as Chen et al. [70] reported (Table 4).

3.4. Methodological Quality

Sixteen studies met the methodological high-quality threshold of 70% (Table 5) [26,50,52,54,56–58,60,62,63,66–70,75]. Four studies scored between 60 and 69% [71,72,74,75], and four studies scored 50–59% [53,59,61,73]. The major source of bias in the resulting 24 papers was the failure to report whether the person(s) experimenting was/were blinded to the study groups and quality controls, followed by the statistical analyses used, such as reporting the confidence interval for change in outcomes from before to after intervention, the distribution of principal confounders in each group of subjects, and adjustment for confounders in the analyses. All studies noticeably described (1) their sample size estimation for each experimental group, (2) their main findings, and (3) the main hypothesis and objectives and validity of the reported main outcome.

Table 5. Quality appraisal of the included studies.

Author	Recruitment /5	Examiner/ 2	Methodology /5	Outcomes/ 2	Missing Data/7	Statistical Analysis/5	Results /2	Overall Score/28	Overall Score 100%
Zhen Sun et al. [26]	4	0	3	2	7	3	2	21	77.7
Kim et al. [50]	4	0	5	2	7	5	2	25	92.5
Nugent et al. [52]	4	0	2	2	7	2	2	19	70.3
Chang et al. [53]	0	0	1	2	7	3	1	14	51.8
Guertin et al. [54]	1	2	5	2	7	5	2	24	88.8
Kim et al. [56]	4	0	4	2	7	5	2	24	88.8
Song et al. [57]	4	0	3	2	7	3	1	20	74.1
Genua et al. [58]	2	0	5	2	6	5	1	20	74.1
D'asheesh et al. [59]	3	0	3	2	4	2	0	14	51.8
Coker et al. [60]	4	0	5	2	7	5	2	25	92.5
Goedert et al. [61]	2	1	2	2	6	2	1	16	59.3
Sinha et al. [62]	2	0	5	2	7	5	2	23	85.2
Clos-Garcia et al. [63]	1	0	5	2	7	5	2	23	81.1
Tan et al. [66]	4	0	5	2	7	3	1	22	81.1
Flemer et al. [67]	4	0	5	2	7	5	2	25	92.6
Zeller et al. [68]	4	0	5	2	7	5	2	25	92.6
Zackular et al. [69]	4	0	5	1	6	3	2	21	77.8

Ohigashi et al. [22]	4	0	3	2	6	1	1	17	62.9
Chen et al. [70]	4	0	3	2	6	4	1	20	74.1
Eklöf et al. [71]	2	0	3	2	6	3	1	17	62.9
Gao et al. [72]	3	0	2	2	7	2	1	17	62.9
Yusuf et al. [73]	3	0	1	2	6	2	1	15	55.5
Weir et al. [74]	4	0	2	2	7	2	1	18	66.7
Yang et al. [75]	4	0	5	2	7	3	2	23	85.2

3.5. Measurement Outcomes

3.5.1. Primary Outcome Measures

Microbial Markers among ADA and CRC Compared to Healthy Control (HC) Using the Untargeted Microbiome Approach

Microbial markers associated with CRC and ADA were evaluated in 18 studies by two approaches: untargeted or targeted method. The untargeted approach applied either 16s rRNA gene or whole genome sequencing analysis, whereas the targeted method used real-time PCR targeting specific microbial genes. Eleven studies used the 16s rRNA gene sequencing analysis [26,50,56,62,63,67,69,70,72,74,75], and two studies used the whole genome sequencing analysis [53,60,68] (Table 3).

There was conflicting evidence of microbial markers between ADA and HC (Nugent et al. [52], Zackular et al. [69], Chen et al. [70], Gao et al. [72]). However, there was strong evidence of associated microbial markers for CRC compared to ADA. Two microbial markers were found to be increased in CRC compared to ADA, *Fusobacterium* spp. (Zeller, et al. [68], Zackular et al [69], and Gao et al [72]) and *Porphyromonas* (Zeller et al. [68] and Zackular et al. [69]. *Fusobacterium* spp. was identified in two high-quality studies (Zeller et al. [68] and Zackular et al. [69]) and one moderate-quality paper (Gao et al. [72]). *Porphyromonas* was profiled in two high-quality papers (Zeller et al. [68], Zackular et al. [69]) (Table 6a).

Table 6. Levels of evidence for summary statements for each intervention.

a. Untargeted Microbiome Identification			
Study (Appraisal Quality)	**Increased in ADA vs. HC**	**Increased in CRC vs. ADA**	**Increased in CRC vs. HC**
Nugent et al. [52] 66.6% (L)	*Bifidobacterium* sp. *Eubacteria*		
Chang et al. [53] 51.8% (L)			*Streptococcus gallolyticus*, *Haemophillus parainfluenza*, *Dialister* sp. *Marseille-P5638*, *Ruthenibacterium lactatiformans*
Kim et al. [56] 88.8% (H)			*Bifidobacterium*, *Collinsella*, *Blautia*, *Lachnoclostridium* Lachnospiraceae, *Dorea* *Eubacterium coprostanoligenes group* Ruminococcaceae-*Ruminococcus* *Faecalibacterium*, *Subdoligranulum* *Catenibacterium*, *Parvimonas* *Ruminiclostridium*, *Enterobacter* *Diaphorobacter*
Sinha et al. [62] 85.2% (H)			*Fusobacterium*, *Porphyromonas* *Clostridia*, Lachnospiraceae
Flemer et al. [67] 92.6% (H)			*Bacteroides*, *Roseburia* *Ruminococcus*, *Oscillibacter* *Porphyromonas*, *Peptostreptococcus*, *Parvimonas*, *Fusobacterium*
Zeller et al. [68] 92.6% (H)		*Fusobacterium nucleatum subsp. vincentii* *Fusobacterium nucleatum subsp. Animalis* *Fusobacterium nucleatum subsp. nucleatum* *Fusobacterium nucleatum subsp. polymorphum* *Porphyromonas asaccharolytica* *Prevotella nigrescens* *Peptostreptococcus stomatis* *Parvimonas sp.* *Parvimonas micra* *Olsenella uli* *Parvimonas sp.* *Streptococcus anginosus*	*Fusobacterium nucleatum subsp. vincentii* *Fusobacterium nucleatum subsp. Animalis* *Fusobacterium nucleatum subsp. nucleatum* *Pseudoflavonifractor capillosus* *Fusobacterium nucleatum subsp. polymorphum* *Porphyromonas asaccharolytica* *Ruminococcaceae bacterium* *Prevotella nigrescens* *Peptostreptococcus stomatis* *Leptotrichia hofstadii* *Parvimonas sp.* *Parvimonas micra* *Bacteroides fragilis* *Bilophila wadsworthia* *Neisseria sp.* *Campylobacter rectus* *Selenomonas sputigena*

							Leptotrichia buccalis *Clostridium hylemonae* *Clostridium symbiosum*			
Zackular et al. [69] 77.8% (H)	Ruminococcaceae *Clostridium* *Pseudomonas* Porphyromonadaceae		*Fusobacterium* *Bacteroides* *Phascolarctobacterium* *Porphyromonas*				*Fusobacterium* *Porphyromonas* *Lachnospiraceae* Enterobacteriaceae			
Chen et al. [70] 74.1 (H)	*Bacteroides* *Escherichia* *Faecalibacterium* *Citrobacter*									
Gao et al. [72] 62.9% (L)	*Bacillus cereus* *Bacillus thuringiensis* *Bacillus amyloliquefaciens* *Cronobacter sakazakii*		*Alcanivorax hongdengensis* *Burkholderia mallei* *Clostridium ramosum* *Coprobacillus Sp* *Fusobacterium sp*				*Streptococcus intermedius* *Peptostreptococcus stomatis* *Parvimonas micra* *F. nucleatum*			
Weir et al. [74] 66.7% (L)							*Acidaminobacter Citrobacter farmer* *Akkermansia muciniphila*			
Yang et al. [75] 85.2% (H)							Enterobacteriaceae *Fusobacterium*			
				Increased in ADA vs. HC						
Overlapping microbial markers				No common microbial markers 4 studies [52,69,70,72]						
Level of evidence					Conflicting					
				Increased in CRC vs. ADA						
Overlapping microbial markers			*Fusobacterium* sp. 3 studies [68,69,72]				*Porphyromonas* *2 studies* [68,69]			
Level of evidence			Strong				Strong			
				Increased in CRC vs. HC						
Overlapping microbial markers	Lachnospiraceae-*Lachnoclostridium* 3 studies [56,62,69]	Ruminococcaceae-*Ruminococcus* 4 studies [56,62,67,68]	*Parvimonas* *Parvimonas micra* 4 studies [56,67,68,72]	Enterobacteriaceae 2 studies [69,75]	*Fusobacterium* sp 5 studies [62,67–69,75]	*Bacteroides* 2 studies [67,68]	*Peptostreptococcus* sp 2 studies [67,72]	*Clostridia* sp *C. hylemonae* *C. symbiosum* 2 studies [62,68]	*Porphyromonas* *4 studies* [62,67–69]	*Streptococcus sp* *S. gallolyticus, S. intermedius* 2 studies [53,72]
Level of evidence	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Limited
				b. Targeted microbiome identification						
	Study (Appraisal quality)			**Increased in ADA vs. HC**		**Increased in CRC vs. ADA**			**Increased in CRC vs. HC**	
	D'asheesh et al. [59]			*Bifidobacterium sp. Eubacteria*					*Enterococcus faecalis*	

51.8 (L)			
Clos-Garcia et al. [63] 81.1% (H)		*Staphylococcus and Parvimonas*	*Fusobacterium*, *Staphylococcus and Parvimonas*
Ohigashi et al. [22] 62.9% (L)			*C. difficile* *C. perfringens*, *Pseudomonas* *,1
Eklöf et al. [71] 62.92% (L)			*F. nucleatum*
Increased in ADA vs. HC			
Overlapping microbial markers		Only one study was reported. [12]	
Level of evidence		NO	
Increased in CRC vs. ADA			
Overlapping microbial markers		Only one study was reported. [63]	
Level of evidence		NO	
Increased in CRC vs. HC			
Overlapping microbial markers		Fusobacterium sp 2 studies [63,71]	
Level of evidence		Moderate	
c. Untargeted Metabolites Identification			
Study (Appraisal quality)	Increased in ADA vs. HC		Increased in CRC vs. HC
Kim et al. [56] 92.5% (H)	<u>Endocannabinoid</u> N acetyl-cadverine Bilirubin ZZ Lionleoyl ethanolamide Oleoyl ethanolamide Palmitoyl ethanolamide 3-Hydroxy-palmitate Myristoleate Palmitoleate 1-Linoleoyl-GPE 1-Palmitioyl -GPE	<u>Polyunsaturated fatty acid</u> Docosahexaenoate Docosapentaenoate Hexadecadienoate	
	<u>Secondary bile acid</u> 3b-Hydroxy-5-cholenoic acid Deoxycholate	<u>Sphingolipid</u> N-palmitoyl-saphinganine Hexadecasphinganine Sphinganine Piperine 3,7-Dimethyl-urate	

Nugent et al. [52] 66.7% (L)	The inflammatory metabolite prostaglandin E2			
Kim et al. [50] 88.8% (H)	<u>Aminoacids</u> Leucine Isoleucine Alanine Lysine Tyramine Aminoisobutyric acid	<u>Amino alcohol</u> Ethanolamine <u>Aromatic alcohol</u> Phenol		
	<u>Carboxylic acid</u> Furoic acid Succinic acid Oxalic acid	<u>Fatty acid</u> Butanoic acid Hexanoic acid Palmitic acid Oleic acid		
Godert et al. [61] 59.3% (L)			<u>Heme-related molecules</u> Heme Z-18565 X_19549	<u>Cofactors. and vitamin</u> α-Tocopherol γ-Tocopherol Pterin
			<u>Xenobiotics</u> 4-Acetamidophenol 2-Hydroxyacetaminophen sulfate 3-Cystein-S-YL-acetaminophen p-Acetamidophenylglucuronide Para-aminobenzoic acid (PABA) N-2-Furoyl-glycine Sitostanol p-Hydroxybenzaldehyde Mandelate	<u>Peptides/Aminoacids</u> Histidine Cis-Urocanate Tryptophyl-glycine Leucyl-tryptophan Alanyl-histidine Histidyl-glycine Tyrosylglutamine Histidyl-alanine Valyl-aspartate Pyro-glutamyl-glycine Alanyl-leucine Alanyl-tryptophan Histidylphenylalanine Leucyl-glutamate Leucyl-serine α-Glutamyl-valine Prolyl-alanine Valyl-histidine
			<u>Lipids</u> Palmitoyl-sphingomyelin Conjugated linoleate-18-2N7 3-Dehydrocarnitine	

Shina et al. [62] 85.5% (H)	Palmitoyl_Sphingomyelin p_Hydroxybenzaldhyde		
Tan et al. [66] 81.1% (H)	Fatty acid metabolism β-hydroxybutyrate betaine Glycerol Oleamide Oleic acid Erythrotetrofuranose Carnitine (18:1) Linolic acid Acetyl carnitine Elaidic acid 3-oxodecanoic acid Palmitic acid	valine, leucine, and isoleucine degradation Allisoleucine	Arginine and proline metabolism Creatinine
	Purine nucleotide synthetics Xanthosine	Cystine & methionine metabolism Cystine	Carbohydrate metabolism Threitol
	Phospholipid metabolism Sphinganine CPA(18:0/0:0)	Glutathione metabolism 2-hydroxybutyric acid 2-aminobutanoic acid TCA cycle Pyruvate Vitamin B6 metabolism Glycolaldehyde	Others Tetrahydrogestrinone Allyl isothiocyanate Proline
Weir et al. [74] 66.7% (L)	Aminoacids Alanine Glutmate Glycine Aspartic acid Leucine Lysine Proline Serine Threonine Valine Phenylalanine		Carboxylic acids Beneneacetic acid Propionic acid Mysteric acid Pantothenic acid
		Steroids Cholesterol derivative	
Yang et al. [75] 85.2% (H)	4-Methylvaleric acid 9-(2-Carboxyethyl)-2,2,4,4-tetramethyl-1,2,3,4-tetrahydro-gamma-carboline Adenosine Butanoic acid d-2-Aminobutyric acid		

			DL-Ornithine D-Proline, n-propoxycarbonyl-, hexadecyl ester Heptanedioic acid Heptanoic acid Hexane, 2,5-dimethyl L-5-Hydroxytryptophan L-Lysine L-Tryptophan L-Norleucine L-Norvaline Pentanoic acid N-Acetyl-D-glucosamine Cadaverine
		Increased in ADA vs. HC	
Overlapping metabolite markers		No common metabolites 5 studies [50,52,56,74,75]	
Level of evidence		Conflicting	
		Increased in CRC vs. HC	
Overlapping metabolite markers	Palmitoyl-sphingomyelin 2 studies [61,62]		Proline 2 studies [66,74]
Level of evidence	Moderate		Moderate
		d. Targeted metabolites identification	
Study (Appraisal Quality)	**Increased in ADA vs. HC**	**Increased in CRC vs. ADA**	**Increased in CRC vs. HC**
Zhen Sun et al. [26] 77.7% (H)	Kynurenin(KYN) Indole-3-aldehyde (IALD) and Indole-3-carboxylic acid (I3CA) The ratio of KYN to Trp (KYN/Trp ratio)		Kynurenin(KYN) Indole-3-aldehyde (IALD) and Indole-3-carboxylic acid (I3CA) The ratio of KYN to Trp (KYN/Trp ratio)
Guertin et al. [54] 88.8% (H)			Serum choline
Song et al. [57] 74.1% (L)			Monounsaturated fatty acids (MUFAs) C18:1ω-9 Oleic acid ω-6 polyunsaturated fatty acids (PUFAs) C18:2ω-6 Linoleic acid
Genua et al. [58] 74.1% (L)			2-MethylButyric Acid Acetic Acid Propionic acids
Coker et al. [60] 92.5% (H)		Phenyllactic acid, Phenylacetic acid, L-Phenylalanine, L-Valine, L-Alpha-aminobutyric acid, L-Proline, L-Alanine	L-alanine, glycine L-proline

			Oxoglutaric acid, L-Isoleucine, Gamma-Aminobutyric acid, L-Leucine, Glycine, L-Methionine, L-Tyrosine, L-Aspartic acid, Butyric acid, Glutathione, Succinic acid, 2-Hydroxybutyric acid, Malic acid, 3-Aminoisobutanoic acid, Ornithine, Beta-Alanine, Myristic acid, Oxoadipic acid, Alpha-Linolenic acid, L-Serine, Nicotinic acid, Linoleic acid, Pelargonic acid, Pyroglutamic acid, Glutaric acid, Hexanoic acid, L-Homoserine, 5-Dodecenoic acid, Pimelic acid	L-aspartic acid L-valine L-leucine L-serine myristic acid phenyl lactic acid oxoglutaric acid L-phenylalanine L-alpha-aminobutyric acid phenylacetic acid palmitoleic acid 3-aminoisobutanoic acid norvaline
Ohigashi et al. [22] 62.9% (M)						Succinic acid
Yusuf et al. [73] 55.5% (M)						The opposite decrease in Acetate Propionate butyrate acids
Increased in ADA vs. HC						
Overlapping microbial markers			Only one study [19]			
Level of evidence			NO			
Increased in CRC vs. ADA						
Overlapping microbial markers			Only one study [60]			
Level of evidence			NO			
Increased in CRC vs. HC						
Overlapping microbial markers			No common metabolites 6 studies [26,54,57,60,73,75]			
Level of evidence			Conflicting			
e. Untargeted microbial markers for tumor stages and locations						
Study (Appraisal Quality)	**Microbial Markers in CRC Early Stage I**	**Microbial Markers in CRC III Stage**	**Microbial Markers in CRC IV, Late Stage**	**Microbial Markers in Distal Cancers vs. Proximal Cancers**	**Microbial Markers in Rectal vs. Proximal Cancers**	**Microbial Markers in Proximal Cancer**
Flemer et al. [67] 92.6% (H)				Alistipes Akkermansia Halomonas Shewanella	Alistipes Akkermansia Halomonas Shewanella	Faecalibacterium Blautia Clostridium
Gao et al. [72] 62.9% (M)	Escherichia/Shigella -	Bacteroides	Saccharibacteria incertaesedis	Escherichia/Shigella		
Microbial markers in CRC early stage I						
Overlapping microbial markers			Only one study reported. [72]			
Level of evidence			NO			
Microbial markers in CRC III stage						

Overlapping microbial markers	Only one study reported. [72]
Level of evidence	NO
Microbial markers in CRC IV, late-stage	
Overlapping microbial markers	Only one study reported. [72]
Level of evidence	NO
Microbial markers in distal cancers vs. proximal cancers	
Overlapping microbial markers	No common metabolites Two studies [67,72]
Level of evidence	Conflicting
Microbial markers in rectal vs. proximal cancers	
Overlapping microbial markers	Only one study reported. [67]
Level of evidence	NO
Microbial markers in proximal cancer	
Overlapping microbial markers	Only one study reported. [67]
Level of evidence	NO

f. Targeted microbial markers for tumor stages and locations

Study (Appraisal Quality)	**Microbial Markers in CRC IV, Late Stage**	**Microbial Markers on Right Side**
Clos-Garcia et al. [63] 81.1% (H)	Bulleidia Fusobacterium Butyrivibrio Peptostreptococcus Staphylococcus Parvimonas Selenomonas	
Ohigashi et al. [22] 62.9% (M)		Clostridium perfringens
Microbial markers in CRC IV, late-stage		
Overlapping microbial markers	Only one study reported. [63]	
Level of evidence	NO	
Microbial markers on right side		
Overlapping microbial markers	Only one study reported. [22]	

Level of evidence	NO
g. Untargeted metabolite markers for tumor stage and location	
Study (Appraisal Quality)	**Microbial Markers in CRC Late Stage IV vs. Early Stage I**
Tan et al. [66] 81.1% (H)	Beta hydroxybuturate
Microbial markers in CRC late stage IV vs. early stage I Tan et	
Overlapping microbial markers	Only one study reported. [66]
Level of evidence	NO

*[1] healthy control included adenoma and non-adenoma participants.

There was strong evidence that nine microbial markers were associated with CRC compared to HC as follows: *Lachnospiraceae-Lachnoclostridium*, Ruminococcaceae-*Ruminococcus*, *Parvimonas* spp., *P micra*, Enterobacteriaceae, *Fusobacterium* spp., *Bacteroides*, *Peptstreptococcus-P. stomatis*, *Clostridia* spp.-*Clostridium hylemonae*, *Clostridium symbiosum*, and *Porphyromonas-P. asaccharolytica* (Table 6a).

Lachnospiraceae-*Lachnoclostridium and* Ruminococcaceae-*Ruminococcus were identified in three high-quality papers:* Kim et al. [56], Sinha et al. [62], and Zackular et al. [69] and Kim et al. [56], Flemer et al. [67] and Zeller, et al. [68], respectively. *Parvimonas* spp.-*P. micra* was profiled in three high-quality studies (Kim et al. [56], Flemer et al. [67], and Zeller et al. [68]) and one in a moderate-quality study (Gao et al. [72]). The group Enterobacteriaceae was found as microbial markers in CRC patients in three high-quality studies (Kim et al. [56], Zackular et al. [69], and Yang et al. [75]) (Table 6a).

Fusobacterium is one of the most common CRC-microbial markers, five high-quality papers (Shina et al. [62] Flemer et al. [67], Zackuler et al. [69] and Yang et al. [75]) and one moderate-quality study (Gao et al. [72]) identified this genus. Zeller et al. [68] typed *Fusombacterium* to the sub-species as *F. nucleatum* subsp. *vincentii*, *F. nucleatum* subsp. *Animalis*, *Fu. nucleatum* subsp. *nucleatum*, *F. nucleatum* subsp. *Polymorphum*, whereas Gao et al. [72] identified the species level only *F. nucleatum* (Table 6a).

Bacteroids were profiled in two high-quality papers (Zeller et al. [68] and Felmer et al. [67]), whereas in Zeller et al. [68] specifically *B. fragilis* was characterized. *P. stomatis* is another CRC-microbial marker that was described in two high-quality studies (Felmer et al. [67] and Zeller et al. [68]) and one low-quality paper (Gao et al. [72]). *Clostridia* spp. was characterized in two high-quality papers (Shinan et al. [62] and Zeller et al. [68]), where two species, *C. hylemonae*, *C. symbiosum*, were described in Zeller et al. [68]. *Porphyromonas* was profiled as a CRC-microbial marker in two high-quality studies (Zeller et al. [68] and Zackular), in Zeller et al. [68] *P. asaccharolytica* was identified (Table 6a).

There was limited evidence of the association of Streptococcus spp. with CRC compared to HC, as the two studies profiled *Streptococcus* spp. were in the low-quality category. Chang et al. [53] identified *S. gallolyticus* and another study (Goa et al. [72]) described S. intermedius. Results indicated no evidence of the association of the other microbial markers shown in Table 6a with CRC compared to HC.

Microbial Markers among ADA and CRC Compared to Healthy Control (HC) Using the Targeted Microbiome Approach

Microbial markers associated with CRC and ADA were evaluated in four studies using real-time PCR targeting specific microbial genes. No studies identified microbial markers associated with ADA compared to HC and ADA compared to CRC. However, there was moderate evidence of *Fusobacterium* spp.-*F. nucleatum* as a microbial marker for CRC compared to HC. Two studies characterized *Fusobacterium* spp. as a microbial marker, one with high-quality (Clos-Garcia et al. [63]) and one with a low-quality score (Eklöf et al. [71]) (Table 6b).

Metabolite Markers among ADA and CRC Compared to Healthy Control (HC) Using the Non-Targeted and Targeted Metabolite Approaches

Metabolite markers linked with CRC and ADA were assessed in 17 studies in two ways, non-targeted or targeted profiling methods. The non-targeted approach applied (1 study [50]) Ultra-Performance Liquid Chromatography/Mass Spectrometry platform (UPLC-MS/MS), (1 study [52]) Liquid chromatography coupled to Gas Chromatography Time-of-Flight Mass Spectrometry (LC-GCTOF-MS/MS), (1 study [56]) Gas Chromatography Time-of-Flight Mass Spectrometry (GCTOF-MS/MS), (1 studies [61]) High-Performance Liquid Chromatography/Mass Spectrometry platform (HLC-MS/MS), (2 studies [74,75]) Gas Chromatography—Mass Spectrometry (GC-MS), (1 study [62]) HPLC-GC-MS/MS analyses, (1 study [66]) GCTOF-MS-UPLC-QTOF-MS, and (1 study [70]) Ion Chromatography/ UPLC-MS/MS. The targeted approach varied among the nine studies: (2

studies [26,63]) UPLC-MS/MS, (1 study [54]) LC-MS/MS, (2 studies [57,74]) GC-MS/MS, (2 studies [58,73]) GC, (1 study [60]) GCTOF-MS/MS, and (1 study [22]) HPLC platforms (Table 3).

There was conflicting evidence of common metabolite markers in ADA compared to HC. Three studies (Kim et al. (high-quality) [56], Nugent et al. (low-quality) [52], and Kim et al. (high-quality) [50]) identified metabolite markers in ADA compared to the HC group using the untargeted means.

There was limited evidence of one metabolite marker (Palmitoyl–sphingomyelin) linked to CRC compared to HC [61,62], whereas there was moderate evidence of another metabolite marker, Proline [66,74], associated with CRC compared to HC. Palmitoyl-sphingomyelin was profiled in two papers, a high-quality paper [62] and a low-quality study [61]. The amino acid, Proline, was identified in a high-quality study [66] and low-quality paper [74] (Table 6c).

Only one study identified metabolite markers using the targeted method for ADA vs. HC groups or ADA vs. CRC groups. Seven studies profiled metabolite markers in CRC vs. HC [26,54,57,58,60,73,75], yet there were conflicting results (no common markers). Three high-quality papers [26,54,60] and four studies of low-quality [57,58,73,75] identified the metabolite markers (Table 6d).

3.5.2. Secondary Outcome Measures

Microbial Markers for Cancer Stages and Locations

Among the included studies, eight papers recorded cancer locations, and nine studies specified cancer stages (Table 3). Based on the untargeted means, one paper [72] identified microbial markers for early stage I, III, and late stage IV. Moreover, one paper [67] profiled microbial markers for different cancer locations. There was no evidence of distinguished microbial markers among the different stages or locations. On the targeted approach, one paper [63] described microbial markers for late-stage IV. Moreover, one paper [22] profiled microbial markers for cancer on the left side. There was no evidence of distinguished microbial markers among the different stages or locations.

4. Discussion

The present systematic review identified strong evidence of two microbial markers for CRC compared to ADA; *Fusobacterium* spp.- *F. nucletaum* (Zelleret al. [68], Zackular et al. [69], and Gao et al. [72]) and *Porphyromonas* (Zeller et al. [68] and Zackular et al. [69]) using the untargeted interventions. Yet, using the targeted method, no evidence was identified for microbial markers associated with CRC compared to ADA.

We identified strong evidence of nine microbial markers associated with CRC compared to HC as follows: Lachnospiraceae-Lachnoclostridium, Ruminococcaceae-*Ruminococcus*, *Parvimona*s spp., *P. micra*, Enterobacteriaceae, *Fusobacterium* spp., *Bacteroides*, *Peptostreptococcus-P. stomatis*, *Clostridia* spp.-*C. hylemonae*, *C. symbiosum*, and *Porphyromonas-P. asaccharolytica* using the untargeted approach. Moreover, results indicated moderate evidence of *Fusobacterium* spp.-*F. nucleatum* as a microbial marker for CRC compared to HC. However, we could not identify evidence for any microbial markers associated with ADA compared to HC using the untargeted and targeted methods.

These findings are consistent with the findings of a systematic review conducted by Russ et al., which investigated the association between the human gut microbiome and the risk of CRC. The study found that *Fusobacterium* and *Bacteroides* were the most enriched microbial species in CRC compared to HC [76]. Another systematic review found nine fecal microbiotas (*Fusobacterium*, *Enterococcus*, *Porphyromonas*, *Salmonell*a, *Pseudomonas*, *Peptostreptococcus*, *Actinomyces*, *Bifidobacterium,* and *Roseburia*) to be associated with colorectal neoplasia [77].

In the current systematic review, results indicated conflicting evidence of metabolite markers for ADA in comparison to HC using the untargeted methods, yet no evidence

using the targeted approach. Limited evidence was demonstrated of Palmitoyl–sphingomyelin as a metabolite marker of CRC compared to HC [61,62], whereas moderate evidence was identified of an amino acid, Proline [66,74], as a metabolite marker for CRC compared to HC using the untargeted approach. However, results demonstrated conflicting evidence of associated metabolite markers with CRC vs. HC using the targeted intervention. There was no evidence of distinguished metabolite markers for ADA compared to CRC using both untargeted and targeted interventions.

The enrichment of amino acids, cadaverine, and creatine in CRC was discovered by a recent meta-analysis that combined LEfSe, random forest (RF), and cooccurrence network approaches to find a collection of global CRC biomarkers. They had a positive correlation with microorganisms linked to CRC (*P. stomatis*, *Gemella morbillorum*, *B. fragilis*, Parvimonas species, *F. nucleatum*, *Solobacterium moorei*, and *Clostridium symbiosum*), but their correlation with microbes linked to controls was negative [6].

Secondary outcomes were not frequently used in the included studies, with no microbial or metabolite fingerprint for the different groups. These included microbial and metabolite markers for cancer stages and cancer locations. Based on the evidence investigated here, no evidence was identified of microbial or metabolite markers for the ADA vs. HC, ADA vs. CRC, or CRC vs. HC using targeted or untargeted interventions. Based on these studies, further investigation of the outcomes in relation to the ADA and CRC is warranted.

5. Study Limitations

Studies only available in English were included in this review; no search of the grey literature was performed. A potential bias in the choice of pertinent studies may have resulted from three sources. As the publications included in this systematic review varied greatly in their methodological approaches, comparison groups, and statistical analyses, meta-analysis was not possible. Gut microbiome and associated metabolites are subjected to confounding variables such as age, gender, diet, medication, smoking, and other lifestyle factors [78]. Moreover, there can be significant differences in the gut microbiome and its metabolites between geographically distinct populations and across countries [79,80].

More than 83% of the included studies focused primarily on identifying biomarkers for CRC diagnosis, yet four studies (16.6%), particularly Sun et al. [26], Nugent et al. [52], Flemer et al. [67], and Yusuf et al. [73], the main aim was to identify microbes or metabolites that could contribute to the pathology of CRC. Sun et al. [26] study identified bacteria and metabolites; Nugent et al. [33] reported associated bacteria with CRC; Flemer et al. [67]; and Yusuf et al. [73] studied only associated metabolites. These papers included healthy controls in comparison to ADA or CRC and performed association analysis to evaluate the contribution of such markers in the CRC progression, suggesting these microbes or metabolites as potential markers of CRC diagnosis. Therefore, we included the four studies in the analysis. However, further evaluation from a diagnostic perspective is much needed.

Various alpha and beta indices, including the Bray–Curtis dissimilarity, Jaccard distance, and UniFrac, as well as the Chao Index, Simpson Index, Shannon Index, ACE Index, and Good's Coverage Index, have been reported across the included research. Most of the studies that were considered demonstrated microbial dysbiosis between CRC and the healthy control group. The stated estimates for alpha and beta diversity are indices rather than true effective difference figures. Due to the non-linear nature of these indices, it is incorrect to compare them between different studies and draw inferences about their biological importance. Therefore, we have not reported and compared these indices in the systematic review.

Most of the included studies were conducted in Asian countries (Table 2), which can be untransferable across the world. Additionally, depending on the interventions used in this research, some of our specific summary statements were in disagreement with one another. (Table 6). There was no consistency in sample types, collection, and storage

temperature. Moreover, the lack of standardization in DNA and metabolite extractions across the included studies has influenced microbiome and metabolite profiling. Further, one of the major conflicts observed was for the intervention approaches, untargeted and targeted methods. Each method applied different analytical means. Microbiome profiling used either 16S rRNA gene or whole genome sequencing for an untargeted approach, or real-time PCR for a targeted approach. Each method has its limitations from the taxonomic analysis perspective [81]. Likewise, metabolite profiling was conducted by a variety of methods. There was significant variation among these methodologies, which could lead to biases and make comparisons between the groups difficult. [82]. Therefore, the level of evidence assessment was classified into two main categories: the untargeted and targeted approaches for each microbial and metabolite profile. There were three studies with low quality (weighted 51.8%, 55.5%, and 59.3% in the summary statement, respectively). This suggests that even a different observation from a low-quality study could substantially alter the strength of the evidence for a given summary conclusion. This might have made it more difficult to distinguish between fingerprint marks left by different groups and caused frequent inconsistencies in evidence summary statements.

6. Conclusions

We identified strong evidence of two microbial markers, *Fusobacterium* spp.-*F. nucletaum* and *Porphyromonas* for ADA vs. CRC, and nine microbial markers *Lachnospiraceae-Lachnoclostridium*, *Ruminococcaceae-Ruminococcus*, *Parvimonas* spp., *P. micr*a, Enterobacteriaceae, *Fusobacterium* spp., *Bacteroides*, *Peptostreptococcus-P. stomatis*, *Clostridia* spp.-*C. hylemonae*, *Clostridium symbiosum*, and *Porphyromonas-P. asaccharolytica* for CRC vs. HC.

Based on the data that have already been reviewed here, there is encouraging evidence that microbial markers from fecal samples may be used to develop new, inexpensive tests that could supplement the collection of existing non-invasive CRC screening tools. However, to make results more comparable and allow for the drawing of conclusions on a wider scale, future research should concentrate on creating standardized and reproducible protocols for researching the human gut microbiota.

The remaining evidence of metabolite markers among the different groups ADA vs. HC, ADA vs. HC, and CRC vs. HC was not of sufficiently high quality to permit further conclusions. With this finding, these microbial markers can be used in a panel for the diagnosis of ADA and CRC. Further research in the metabolite markers area is needed to evaluate the possibility of diagnostic or prognostic markers for colorectal cancer.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microorganisms11082037/s1, Supplementary material of this systematic review can be found in the online version. The following supplementary material of this systematic review can be found in the online version. Table S1. Extraction form, Table S2. Appraisal quality form.

Author Contributions: Conceptional design of the project, writing and editing the manuscript, and generating figures and tables were performed by A.A.A. and R.M.A. (Renad M. Alhamawi); W.M.-S., H.A.A., R.M.A. (Renad M. Alhamawi), Y.A.A., and W.M.-S. wrote and reviewed the manuscript. Five authors (Y.A.A., R.M.A. (Reema M. Almisned), H.A.A., S.M.K., and A.A.J.) performed the search and initial evaluation and extracted the abstracts and full text. All authors have read and agreed to the published version of the manuscript.

Funding: This research received a grant from the Ministry of Education in Saudi Arabia through project number 422/12.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia, for funding this research work through project

number 422/12. Also, the authors would like to extend their appreciation to Taibah University for its supervision support.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Xi, Y.; Xu, P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl. Oncol.* **2021**, *14*, 101174.
2. McGuire, G. *Switzerland: World Health Organization, International Agency for Research on Cancer*; World Health Organization: Geneva, Switzerland, 2015.
3. Ferlay,J.; Ervik, M.; Lam, F.; Colombet, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer, 2020. Available online: https://gco.iarc.fr/today (accessed on 20 April 2023).
4. Wild, C.P.; Stewart, B.W.; Wild, C. *World Cancer Report 2014*; World Health Organization: Geneva, Switzerland, 2014.
5. Thursby, E.; Juge, N. Introduction to the human gut microbiota. *Biochem. J.* **2017**, *474*, 1823–1836.
6. Avuthu, N.; Guda, C. Meta-Analysis of Altered Gut Microbiota Reveals Microbial and Metabolic Biomarkers for Colorectal Cancer. *Microbiol. Spectr.* **2022**, *10*, e00013-22.
7. Tjalsma, H.; Boleij, A.; Marchesi, J.R.; Dutilh, B.E. A bacterial driver–passenger model for colorectal cancer: Beyond the usual suspects. *Nat. Rev. Microbiol.* **2012**, *10*, 575–582.
8. Tilg, H.; Adolph, T.E.; Gerner, R.R.; Moschen, A.R. The intestinal microbiota in colorectal cancer. *Cancer Cell* **2018**, *33*, 954–964.
9. Yu, J.; Feng, Q.; Wong, S.H.; Zhang, D.; yi Liang, Q.; Qin, Y.; Tang, L.; Zhao, H.; Stenvang, J.; Li, Y. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut* **2017**, *66*, 70–78.
10. Torre, L.A.; Bray, F.; Siegel, R.L.; Ferlay, J.; Lortet-Tieulent, J.; Jemal, A. Global cancer statistics, 2012. *CA: A Cancer J. Clin.* **2015**, *65*, 87–108.
11. Stracci, F.; Zorzi, M.; Grazzini, G. Colorectal cancer screening: Tests, strategies, and perspectives. *Front. Public Health* **2014**, *2*, 210.
12. Lagier, J.-C.; Dubourg, G.; Million, M.; Cadoret, F.; Bilen, M.; Fenollar, F.; Levasseur, A.; Rolain, J.-M.; Fournier, P.-E.; Raoult, D. Culturing the human microbiota and culturomics. *Nat. Rev. Microbiol.* **2018**, *16*, 540–550.
13. Boleij, A.; Hechenbleikner, E.M.; Goodwin, A.C.; Badani, R.; Stein, E.M.; Lazarev, M.G.; Ellis, B.; Carroll, K.C.; Albesiano, E.; Wick, E.C. The Bacteroides fragilis toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clin. Infect. Dis.* **2015**, *60*, 208–215.
14. Zuppi, C.; Messana, I.; Tapanainen, P.; Knip, M.; Vincenzoni, F.; Giardina, B.; Nuutinen, M. Proton nuclear magnetic resonance spectral profiles of urine from children and adolescents with type 1 diabetes. *Clin. Chem.* **2002**, *48*, 660–662.
15. Emaus, A.; Veierød, M.B.; Tretli, S.; Finstad, S.E.; Selmer, R.; Furberg, A.S.; Bernstein, L.; Schlichting, E.; Thune, I. Metabolic profile, physical activity, and mortality in breast cancer patients. *Breast Cancer Res. Treat.* **2010**, *121*, 651–660.
16. Parmentier-Decrucq, E.; Duhamel, A.; Ernst, O.; Fermont, C.; Louvet, A.; Vernier-Massouille, G.; Cortot, A.; Colombel, J.F.; Desreumaux, P.; Peyrin-Biroulet, L. Effects of infliximab therapy on abdominal fat and metabolic profile in patients with Crohn's disease. *Inflamm. Bowel Dis.* **2009**, *15*, 1476–1484.
17. Murdoch, T.B.; Fu, H.; MacFarlane, S.; Sydora, B.C.; Fedorak, R.N.; Slupsky, C.M. Urinary metabolic profiles of inflammatory bowel disease in interleukin-10 gene-deficient mice. *Anal. Chem.* **2008**, *80*, 5524–5531.
18. Williams, H.R.; Willsmore, J.D.; Cox, I.J.; Walker, D.G.; Cobbold, J.F.; Taylor-Robinson, S.D.; Orchard, T.R. Serum metabolic profiling in inflammatory bowel disease. *Dig. Dis. Sci.* **2012**, *57*, 2157–2165.
19. Fliss-Isakov, N.; Zelber-Sagi, S.; Webb, M.; Halpern, Z.; Shibolet, O.; Kariv, R. Distinct metabolic profiles are associated with colorectal adenomas and serrated polyps. *Obesity* **2017**, *25*, S72–S80.
20. Farshidfar, F.; Weljie, A.M.; Kopciuk, K.; Buie, W.D.; MacLean, A.; Dixon, E.; Sutherland, F.R.; Molckovsky, A.; Vogel, H.J.; Bathe, O.F. Serum metabolomic profile as a means to distinguish stage of colorectal cancer. *Genome Med.* **2012**, *4*, 42.
21. Ni, Y.; Xie, G.; Jia, W. Metabonomics of human colorectal cancer: New approaches for early diagnosis and biomarker discovery. *J. Proteome Res.* **2014**, *13*, 3857–3870.
22. Ohigashi, S.; Sudo, K.; Kobayashi, D.; Takahashi, O.; Takahashi, T.; Asahara, T.; Nomoto, K.; Onodera, H. Changes of the intestinal microbiota, short chain fatty acids, and fecal pH in patients with colorectal cancer. *Dig. Dis. Sci.* **2013**, *58*, 1717–1726.
23. Barberini, L.; Restivo, A.; Noto, A.; Deidda, S.; Fattuoni, C.; Fanos, V.; Saba, L.; Zorcolo, L.; Mussap, M. A gas chromatography-mass spectrometry (GC-MS) metabolomic approach in human colorectal cancer (CRC): The emerging role of monosaccharides and amino acids. *Ann. Transl. Med.* **2019**, *7*, 727.
24. Liu, T.; Song, X.; Khan, S.; Li, Y.; Guo, Z.; Li, C.; Wang, S.; Dong, W.; Liu, W.; Wang, B. The gut microbiota at the intersection of bile acids and intestinal carcinogenesis: An old story, yet mesmerizing. *Int. J. Cancer* **2020**, *146*, 1780–1790.
25. Yu, S.; Shao, X.; Zhou, Y.; Yu, Y.; Kuai, X.; Zhou, C. Bidirectional regulation of bile acid on colorectal cancer through bile acid-gut microbiota interaction. *Am. J. Transl. Res.* **2021**, *13*, 10994.
26. Sun, X.-Z.; Zhao, D.-Y.; Zhou, Y.-C.; Wang, Q.-Q.; Qin, G.; Yao, S.-K. Alteration of fecal tryptophan metabolism correlates with shifted microbiota and may be involved in pathogenesis of colorectal cancer. *World J. Gastroenterol.* **2020**, *26*, 7173.

27. Jalandra, R.; Dalal, N.; Yadav, A.K.; Verma, D.; Sharma, M.; Singh, R.; Khosla, A.; Kumar, A.; Solanki, P.R. Emerging role of trimethylamine-N-oxide (TMAO) in colorectal cancer. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 7651–7660.
28. Higgins, J.P.T.; Thomas, J.; Chandler, J.; Cumpston, M.; Li, T.; Page, M.J. In *Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (Updated February 2022);* Welch, V.A., Eds.; Cochrane: London, UK, 2022. Available online: www.training.cochrane.org/handbook (accessed on 20 April 2023).
29. Liberati, A.; Altman, D.G.; Tetzlaff, J.; Mulrow, C.; Gøtzsche, P.C.; Ioannidis, J.P.; Clarke, M.; Devereaux, P.J.; Kleijnen, J.; Moher, D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *Ann. Intern. Med.* **2009**, *151*, W65–W94. https://doi.org/10.7326/0003-4819-151-4-200908180-00136.
30. Vandenbroucke, J.P.; Elm, E.v.; Altman, D.G.; Gøtzsche, P.C.; Mulrow, C.D.; Pocock, S.J.; Poole, C.; Schlesselman, J.J.; Egger, M.; Initiative, S. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and elaboration. *Ann. Intern. Med.* **2007**, *147*, W163–W194. Available online: https://www.acpjournals.org/doi/10.7326/0003-4819-147-8-200710160-00010-w1 (accessed on 20 April 2023).
31. Cornelius, L.; Van der Klink, J.; Groothoff, J.; Brouwer, S. Prognostic factors of long term disability due to mental disorders: A systematic review. *J. Occup. Rehabil.* **2011**, *21*, 259–274.
32. Wang, Q.; Li, L.; Xu, R. A systems biology approach to predict and characterize human gut microbial metabolites in colorectal cancer. *Sci. Rep.* **2018**, *8*, 6225.
33. Konishi, Y.; Okumura, S.; Matsumoto, T.; Itatani, Y.; Nishiyama, T.; Okazaki, Y.; Shibutani, M.; Ohtani, N.; Nagahara, H.; Obama, K. Development and evaluation of a colorectal cancer screening method using machine learning-based gut microbiota analysis. *Cancer Med.* **2022**, *11*, 3194–3206.
34. Iwasaki, M.; Kanehara, R.; Yamaji, T.; Katagiri, R.; Mutoh, M.; Tsunematsu, Y.; Sato, M.; Watanabe, K.; Hosomi, K.; Kakugawa, Y. Association of Escherichia coli containing polyketide synthase in the gut microbiota with colorectal neoplasia in Japan. *Cancer Sci.* **2022**, *113*, 277–286.
35. Jin, Y.; Liu, Y.; Zhao, L.; Zhao, F.; Feng, J.; Li, S.; Chen, H.; Sun, J.; Zhu, B.; Geng, R. Gut microbiota in patients after surgical treatment for colorectal cancer. *Environ. Microbiol.* **2019**, *21*, 772–783.
36. Chen, F.; Dai, X.; Zhou, C.-C.; Li, K.-x.; Zhang, Y.-j.; Lou, X.-Y.; Zhu, Y.-M.; Sun, Y.-L.; Peng, B.-X.; Cui, W. Integrated analysis of the faecal metagenome and serum metabolome reveals the role of gut microbiome-associated metabolites in the detection of colorectal cancer and adenoma. *Gut* **2022**, *71*, 1315–1325.
37. Sugimura, N.; Li, Q.; Chu, E.S.H.; Lau, H.C.H.; Fong, W.; Liu, W.; Liang, C.; Nakatsu, G.; Su, A.C.Y.; Coker, O.O. Lactobacillus gallinarum modulates the gut microbiota and produces anti-cancer metabolites to protect against colorectal tumourigenesis. *Gut* **2022**, *71*, 2011–2021.
38. Sánchez-Alcoholado, L.; Laborda-Illanes, A.; Otero, A.; Ordóñez, R.; González-González, A.; Plaza-Andrades, I.; Ramos-Molina, B.; Gómez-Millán, J.; Queipo-Ortuño, M.I. Relationships of gut microbiota composition, short-chain fatty acids and polyamines with the pathological response to neoadjuvant radiochemotherapy in colorectal cancer patients. *Int. J. Mol. Sci.* **2021**, *22*, 9549.
39. Yu, S.Y.; Xie, Y.H.; Qiu, Y.W.; Chen, Y.X.; Fang, J.Y. Moderate alteration to gut microbiota brought by colorectal adenoma resection. *J. Gastroenterol. Hepatol.* **2019**, *34*, 1758–1765.
40. Bi, D.; Zhu, Y.; Gao, Y.; Li, H.; Zhu, X.; Wei, R.; Xie, R.; Wei, Q.; Qin, H. A newly developed PCR-based method revealed distinct Fusobacterium nucleatum subspecies infection patterns in colorectal cancer. *Microb. Biotechnol.* **2021**, *14*, 2176–2186.
41. Ou, J.; Carbonero, F.; Zoetendal, E.G.; DeLany, J.P.; Wang, M.; Newton, K.; Gaskins, H.R.; O'Keefe, S.J. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am. J. Clin. Nutr.* **2013**, *98*, 111–120.
42. Hester, C.M.; Jala, V.R.; Langille, M.G.; Umar, S.; Greiner, K.A.; Haribabu, B. Fecal microbes, short chain fatty acids, and colorectal cancer across racial/ethnic groups. *World J. Gastroenterol. WJG* **2015**, *21*, 2759.
43. Guo, Y.-P.; Shao, L.; Wang, L.; Chen, M.-Y.; Zhang, W.; Huang, W.-H. Bioconversion variation of ginsenoside CK mediated by human gut microbiota from healthy volunteers and colorectal cancer patients. *Chin. Med.* **2021**, *16*, 28.
44. Xu, R.; Wang, Q.; Li, L. A genome-wide systems analysis reveals strong link between colorectal cancer and trimethylamine N-oxide (TMAO), a gut microbial metabolite of dietary meat and fat. *BMC Genom.* **2015**, *16*, S4.
45. Okumura, S.; Konishi, Y.; Narukawa, M.; Sugiura, Y.; Yoshimoto, S.; Arai, Y.; Sato, S.; Yoshida, Y.; Tsuji, S.; Uemura, K. Gut bacteria identified in colorectal cancer patients promote tumourigenesis via butyrate secretion. *Nat. Commun.* **2021**, *12*, 5674.
46. Tang, Q.; Cang, S.; Jiao, J.; Rong, W.; Xu, H.; Bi, K.; Li, Q.; Liu, R. Integrated study of metabolomics and gut metabolic activity from ulcerative colitis to colorectal cancer: The combined action of disordered gut microbiota and linoleic acid metabolic pathway might fuel cancer. *J. Chromatogr. A* **2020**, *1629*, 461503.
47. Katsidzira, L.; Ocvirk, S.; Wilson, A.; Li, J.; Mahachi, C.; Soni, D.; DeLany, J.; Nicholson, J.; Zoetendal, E.; O'keefe, S. Differences in fecal gut microbiota, short-chain fatty acids and bile acids link colorectal cancer risk to dietary changes associated with urbanization among Zimbabweans. *Nutr. Cancer* **2019**, *71*, 1313–1324.
48. Ocvirk, S.; Wilson, A.S.; Posma, J.M.; Li, J.V.; Koller, K.R.; Day, G.M.; Flanagan, C.A.; Otto, J.E.; Sacco, P.E.; Sacco, F.D. A prospective cohort analysis of gut microbial co-metabolism in Alaska Native and rural African people at high and low risk of colorectal cancer. *Am. J. Clin. Nutr.* **2020**, *111*, 406–419.
49. Ai, D.; Pan, H.; Li, X.; Wu, M.; Xia, L.C. Association network analysis identifies enzymatic components of gut microbiota that significantly differ between colorectal cancer patients and healthy controls. *PeerJ* **2019**, *7*, e7315.

50. Kim, M.; Vogtmann, E.; Ahlquist, D.A.; Devens, M.E.; Kisiel, J.B.; Taylor, W.R.; White, B.A.; Hale, V.L.; Sung, J.; Chia, N. Fecal metabolomic signatures in colorectal adenoma patients are associated with gut microbiota and early events of colorectal cancer pathogenesis. *mBio* **2020**, *11*, e03186-19.
51. Ahlquist, D.A.; Sargent, D.J.; Loprinzi, C.L.; Levin, T.R.; Rex, D.K.; Ahnen, D.J.; Knigge, K.; Lance, M.P.; Burgart, L.J.; Hamilton, S.R. Stool DNA and occult blood testing for screen detection of colorectal neoplasia. *Ann. Intern. Med.* **2008**, *149*, 441–450.
52. Nugent, J.L.; McCoy, A.N.; Addamo, C.J.; Jia, W.; Sandler, R.S.; Keku, T.O. Altered tissue metabolites correlate with microbial dysbiosis in colorectal adenomas. *J. Proteome Res.* **2014**, *13*, 1921–1929.
53. Chang, H.; Mishra, R.; Cen, C.; Tang, Y.; Ma, C.; Wasti, S.; Wang, Y.; Ou, Q.; Chen, K.; Zhang, J. Metagenomic analyses expand bacterial and functional profiling biomarkers for colorectal cancer in a Hainan cohort, China. *Curr. Microbiol.* **2021**, *78*, 705–712.
54. Guertin, K.A.; Li, X.S.; Graubard, B.I.; Albanes, D.; Weinstein, S.J.; Goedert, J.J.; Wang, Z.; Hazen, S.L.; Sinha, R. Serum Trimethylamine N-oxide, Carnitine, Choline, and Betaine in Relation to Colorectal Cancer Risk in the Alpha Tocopherol, Beta Carotene Cancer Prevention StudySerum TMAO, Related Metabolites, and Colorectal Cancer Risk. *Cancer Epidemiol. Biomark. Prev.* **2017**, *26*, 945–952.
55. Group ACPS. The alpha-tocopherol, beta-carotene lung cancer prevention study: Design, methods, participant characteristics, and compliance. *Ann. Epidemiol.* **1994**, *4*, 1–10.
56. Kim, D.J.; Yang, J.; Seo, H.; Lee, W.H.; Ho Lee, D.; Kym, S.; Park, Y.S.; Kim, J.G.; Jang, I.-J.; Kim, Y.-K. Colorectal cancer diagnostic model utilizing metagenomic and metabolomic data of stool microbial extracellular vesicles. *Sci. Rep.* **2020**, *10*, 2860.
57. Song, E.M.; Byeon, J.-S.; Lee, S.M.; Yoo, H.J.; Kim, S.J.; Lee, S.-H.; Chang, K.; Hwang, S.W.; Yang, D.-H.; Jeong, J.-Y. Fecal fatty acid profiling as a potential new screening biomarker in patients with colorectal cancer. *Dig. Dis. Sci.* **2018**, *63*, 1229–1236.
58. Genua, F.; Mirković, B.; Mullee, A.; Levy, M.; Gallagher, W.M.; Vodicka, P.; Hughes, D.J. Association of circulating short chain fatty acid levels with colorectal adenomas and colorectal cancer. *Clin. Nutr. ESPEN* **2021**, *46*, 297–304.
59. D'asheesh, T.i.A.; Hussen, B.M.; Al-Marzoqi, A.H.; Ghasemian, A. Assessment of oncogenic role of intestinal microbiota in colorectal cancer patients. *J. Gastrointest. Cancer* **2021**, *52*, 1016–1021.
60. Coker, O.O.; Liu, C.; Wu, W.K.K.; Wong, S.H.; Jia, W.; Sung, J.J.; Yu, J. Altered gut metabolites and microbiota interactions are implicated in colorectal carcinogenesis and can be non-invasive diagnostic biomarkers. *Microbiome* **2022**, *10*, 35.
61. Goedert, J.J.; Sampson, J.N.; Moore, S.C.; Xiao, Q.; Xiong, X.; Hayes, R.B.; Ahn, J.; Shi, J.; Sinha, R. Fecal metabolomics: Assay performance and association with colorectal cancer. *Carcinogenesis* **2014**, *35*, 2089–2096.
62. Sinha, R.; Ahn, J.; Sampson, J.N.; Shi, J.; Yu, G.; Xiong, X.; Hayes, R.B.; Goedert, J.J. Fecal microbiota, fecal metabolome, and colorectal cancer interrelations. *PLoS ONE* **2016**, *11*, e0152126.
63. Clos-Garcia, M.; Garcia, K.; Alonso, C.; Iruarrizaga-Lejarreta, M.; D'Amato, M.; Crespo, A.; Iglesias, A.; Cubiella, J.; Bujanda, L.; Falcón-Pérez, J.M. Integrative analysis of fecal metagenomics and metabolomics in colorectal cancer. *Cancers* **2020**, *12*, 1142.
64. Cubiella, J.; Clos-Garcia, M.; Alonso, C.; Martinez-Arranz, I.; Perez-Cormenzana, M.; Barrenetxea, Z.; Berganza, J.; Rodríguez-Llopis, I.; D'amato, M.; Bujanda, L. Targeted UPLC-MS metabolic analysis of human faeces reveals novel low-invasive candidate markers for colorectal cancer. *Cancers* **2018**, *10*, 300.
65. Cubiella, J.; Vega, P.; Salve, M.; Díaz-Ondina, M.; Alves, M.T.; Quintero, E.; Álvarez-Sánchez, V.; Fernández-Bañares, F.; Boadas, J.; Campo, R. Development and external validation of a faecal immunochemical test-based prediction model for colorectal cancer detection in symptomatic patients. *BMC Med.* **2016**, *14*, 128.
66. Tan, B.; Qiu, Y.; Zou, X.; Chen, T.; Xie, G.; Cheng, Y.; Dong, T.; Zhao, L.; Feng, B.; Hu, X. Metabonomics identifies serum metabolite markers of colorectal cancer. *J. Proteome Res.* **2013**, *12*, 3000–3009.
67. Flemer, B.; Lynch, D.B.; Brown, J.M.; Jeffery, I.B.; Ryan, F.J.; Claesson, M.J.; O'Riordain, M.; Shanahan, F.; O'Toole, P.W. Tumour-associated and non-tumour-associated microbiota in colorectal cancer. *Gut* **2017**, *66*, 633–643.
68. Zeller, G.; Tap, J.; Voigt, A.Y.; Sunagawa, S.; Kultima, J.R.; Costea, P.I.; Amiot, A.; Böhm, J.; Brunetti, F.; Habermann, N. Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol. Syst. Biol.* **2014**, *10*, 766.
69. Zackular, J.P.; Rogers, M.A.; Ruffin IV, M.T.; Schloss, P.D. The human gut microbiome as a screening tool for colorectal cancer. *Cancer Prev. Res.* **2014**, *7*, 1112–1121.
70. Chen, C.; Niu, M.; Pan, J.; Du, N.; Liu, S.; Li, H.; He, Q.; Mao, J.; Duan, Y.; Du, Y. Bacteroides, butyric acid and t10, c12-CLA changes in colorectal adenomatous polyp patients. *Gut Pathog.* **2021**, *13*, 1.
71. Eklöf, V.; Löfgren-Burström, A.; Zingmark, C.; Edin, S.; Larsson, P.; Karling, P.; Alexeyev, O.; Rutegård, J.; Wikberg, M.L.; Palmqvist, R. Cancer-associated fecal microbial markers in colorectal cancer detection. *Int. J. Cancer* **2017**, *141*, 2528–2536.
72. Gao, R.; Wang, Z.; Li, H.; Cao, Z.; Gao, Z.; Chen, H.; Zhang, X.; Pan, D.; Yang, R.; Zhong, H. Gut microbiota dysbiosis signature is associated with the colorectal carcinogenesis sequence and improves the diagnosis of colorectal lesions. *J. Gastroenterol. Hepatol.* **2020**, *35*, 2109–2121.
73. Yusuf, F.; Adewiah, S.; Fatchiyah, F. The level short chain fatty acids and HSP 70 in colorectal cancer and non-colorectal cancer. *Acta Inform. Med.* **2018**, *26*, 160.
74. Weir, T.L.; Manter, D.K.; Sheflin, A.M.; Barnett, B.A.; Heuberger, A.L.; Ryan, E.P. Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. *PLoS ONE* **2013**, *8*, e70803.
75. Yang, Y.; Misra, B.B.; Liang, L.; Bi, D.; Weng, W.; Wu, W.; Cai, S.; Qin, H.; Goel, A.; Li, X. Integrated microbiome and metabolome analysis reveals a novel interplay between commensal bacteria and metabolites in colorectal cancer. *Theranostics* **2019**, *9*, 4101.
76. Russ, C.A.; Zertalis, N.A.; Nanton, V. Gut Bacterial Microbiome Profiles Associated with Colorectal Cancer Risk: A Systematic Review and Meta-Analysis. *medRxiv* **2021**, 21258404. https://doi.org/10.1101/2021.06.05.21258404.

77. Yu, L.; Zhao, G.; Wang, L.; Zhou, X.; Sun, J.; Li, X.; Zhu, Y.; He, Y.; Kofonikolas, K.; Bogaert, D. A systematic review of microbial markers for risk prediction of colorectal neoplasia. *Br. J. Cancer* **2022**, *126*, 1318–1328.
78. Poore, G.D.; Kopylova, E.; Zhu, Q.; Carpenter, C.; Fraraccio, S.; Wandro, S.; Kosciolek, T.; Janssen, S.; Metcalf, J.; Song, S.J. Microbiome analyses of blood and tissues suggest cancer diagnostic approach. *Nature* **2020**, *579*, 567–574.
79. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* **2012**, *486*, 207–214.
80. Mancabelli, L.; Milani, C.; Lugli, G.A.; Turroni, F.; Ferrario, C.; van Sinderen, D.; Ventura, M. Meta-analysis of the human gut microbiome from urbanized and pre-agricultural populations. *Environ. Microbiol.* **2017**, *19*, 1379–1390.
81. Durazzi, F.; Sala, C.; Castellani, G.; Manfreda, G.; Remondini, D.; De Cesare, A. Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. *Sci. Rep.* **2021**, *11*, 3030.
82. Breitwieser, F.P.; Lu, J.; Salzberg, S.L. A review of methods and databases for metagenomic classification and assembly. *Brief. Bioinform.* **2019**, *20*, 1125–1136.