Lead Article

Probiotics supplementation in patients with colorectal cancer: a systematic review of randomized controlled trials

Ifeoma Julieth Dikeocha, Abdelkodose Mohammed Al-Kabsi, Eltayeb E.M. Eid, Salasawati Hussin, and Mohammed Abdullah Alshawsh

Context: *Colorectal cancer (CRC) is a leading cause of cancer deaths. Recently, much attention has been given to the microbiome and probiotics as preventive and therapeutic approaches to CRC and the mechanisms involved.* ***Objectives:*** *To interpret the findings of randomized controlled trials (RCTs) of probiotics relative to patients with CRC and to outline challenges of and future directions for using probiotics in the management and prevention of CRC.* ***Data sources:*** *Web of Science, PubMed, ProQuest, Wile,y and Scopus databases were searched systematically from January 17–20, 2020, in accordance with PRISMA guidelines.* ***Study selection:*** *Primacy RCTs that reported the effects of administration to patients with CRC of a probiotic vs a placebo were eligible to be included.* ***Data Extraction:*** *The studies were screened and selected independently by 2 authors on the basis of prespecified inclusion and exclusion criteria. The data extraction and risk-of-bias assessment were also performed independently by 2 authors.* ***Results:*** *A total of 23 RCTs were eligible for inclusion. Probiotics supplementation in patients with CRC improved their quality of life, enhanced gut microbiota diversity, reduced postoperative infection complications, and inhibited pro-inflammatory cytokine production. The use of certain probiotics in patients with CRC also reduced the side effects of chemotherapy, improved the outcomes of surgery, shortened hospital stays, and decreased the risk of death.* Bifidobacteria *and* Lactobacillus *were the common probiotics used across all studies.* ***Conclusion:*** *Probiotics have beneficial effects in patients with CRC regardless of the stage of cancer. There is an opportunity for probiotics to be used in mainstream health care as a therapy in the fight against CRC, especially in early stages; however, larger clinical trialsof selected or a cocktail of probiotics are needed to confirm the efficacy, dosage, and interactions with chemotherapeutics agents.* ***Systematic Review Registration:*** *PROSPERO registration no. CRD42020166865.*

Affiliation: Affiliation: I.J. Dikeocha, A.M. Al-Kabsi, and S. Hussin are with the Faculty of Medicine, University of Cyberjaya, Cyberjaya, Selangor, Malaysia. E.E.M. Eid is with the Department of Pharmaceutical Chemistry and Pharmacognosy, Unaizah College of Pharmacy, Qassim University, Unaizah, Saudi Arabia. M.A. Alshawsh is with the Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Correspondence: Correspondence: M.A. Alshawsh, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. E-mail: alshaweshmam@um.edu.my.

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INTRODUCTION

The World Health Organization estimates that by the year 2030, there will be approximately 27 million new diagnosed cases of colorectal cancer (CRC) worldwide and up to 18 million deaths as a result of CRC; >74 million people are living with the disease.[1] Colorectal cancer starts as a malignant tumor on the inner wall of the colon or rectum of an individual and develops over a long time. The pathological development of CRC progresses from normal epithelium to adenomatoid polyps and eventually to adenocarcinoma.[2] Colorectal cancer is associated with different risk factors, including excessive consumption of unhealthy foods, poor diet, history of polyps, advanced age, excessive smoking, and an unhealthy lifestyle in general. The diseasealso can be a result of genetic disorders and inherited CRC genes.[3] Dysbiosis of the gut microbiota, dysfunction of the immune system, and chronic inflammation might contribute, to some extent, to the pathogenesis of CRC.[4,5] Colorectal cancer is usually categorized as inflammatory, sporadic, or hereditary on the basis of the causative factors and pathogenesis.[6] With better screening and advanced diagnostic procedures available, younger individuals (<20 years) have also been diagnosed with CRC. Although there has been significant progress in development of CRC treatment, such as immunotherapy, survival rates are still quite poor and the severe side effects of treatment procedures like chemotherapy, radiotherapy, and surgery make it difficult to treat and prolong recovery.[7]

The concept of probiotics began in the 20th century and was pioneered by Elie Metchnikoff, a Nobel laureate from Russia, who found that some bacterial strains in humans were beneficial and important for several physiological activities of the human body.[8] Probiotics are generally referred to as prolife microorganisms and are described by the Food and Agriculture Organization of the United Nations and by the World Health Organization as "live microorganisms which when consumed in adequate amounts confer a health effect on the host."[9] Most probiotics belong to a group of lactic acid–producing bacteria, including species of *Propionibacterium*, *Bifidobacterium, Streptococcus*, and *Lactobacillus*. Some probiotic strainshave nonhuman origins, such as those used to ferment dairy products; those of human origin are isolated from human intestine and feces. Several bacterial genera, such as *Streptococcus*, *Bacillus*, and *Enterococcus*, contain species known for probiotic potential, though uncertainties regarding the safety of such probiotics have been raised because, in these genera, there are many pathogenic species, particularly *Enterococcus*.[8] Microorganisms that are not bacteria, like *Saccharomyces* yeasts, are also widely used as probiotics. Several beneficial effects of consumption of probiotics have been documented. The favorable influence of probiotic bacteria refers mainly to their effect on composition of the gut microbiota, their ability to arrive alive in the intestine when administered orally, to modulate the immune system, and to lessen the nonbeneficial bacteria found in the gut by competing for adhesion on the cells of the host, growth factors, and nutrients needed for survival.[10]

On the other hand, prebiotics have been redefined by the International Scientific Association for Probiotics and Prebiotics as "a substrate that is selectively utilized by host microorganisms to confer health benefit."[11] Most prebiotics are nondigestible oligosaccharides, including inulin-type soy oligosaccharides, fructans, and xylooligosaccharides. Prebiotics work by altering the constitution and activity of intestinal microbiota, and they discriminately prompt the development and action of probiotic microorganisms.[12]

The combination of suitable prebiotics with probiotics to form synbiotics promotes the viability of probiotic microorganisms in the gut.[12,13] If prebiotics and probiotics are combined, they not only by enhancing the growth of existing probiotics in the colon but also often encourage the survival, implantation, and development of probiotic strains that are newly introduced to the gut microbiota.[14] Short-chain fatty acids (SCFAs) are produced when prebiotics are fermented, which leads to an increase in probiotic growth. Butyric acid, acetic acid, and propionic acid are the most studied SCFAs; they inhibit the development of CRC by stimulating cell apoptosis and inhibiting cell proliferation.[15,16] In addition, SCFAs arrest cellular growth of cancerous cells through a mechanism of fluctuations in the expression of cell cycle regulators p21 and CB1.[17] Short-chain fatty acids such as butyrate have been seen to decrease the inflammation associated with progression of tumors. For instance, Perrin et al[18] found that butyrate upregulated the expression of intercellular adhesion molecule-1 in PROb rat colon cancer cells. Butyrate is also reported to reduce decay-accelerating factor (DAF) activation in many colon cancer cell lines in which DAF is a deterrent to the removal of cancer cells by complement activation on the surface of the cell membrane.[19]

Preclinical studies of probiotics as an intervention in rats with CRC induced by azoxymethane or 1,2 dimethylhydrazine showed that supplementation of probiotics in higher doses, especially species in the *Lactobacillus* genus (namely, *fermentum*, *plantarum*, *acidophilus*, *casei*, *rhamnosus*, *delbrueckii*, and *gasseri*) were effective in preventing and ameliorating the development of intestinal tumors, preneoplastic lesions, and aberrant crypt foci in the rat model, as well as

decreasing the expression of pro-inflammatory markers that cause intestinal inflammation.[20] In reviews, the use of probiotics by patients with CRC has been reported to have positive effects, including the treatment of chemotherapy-induced diarrhea,[21] reduction of total cholesterol in the serum,[22] and reduction of postoperative complications in patients undergoing colorectal surgery.[23] These studies, however, focused mainly on singular outcome and did not provide a comprehensive overview on the probiotics' function in CRC management and the potential mechanistic pathways in prevention and treatment of CRC.

OBJECTIVES

Our objective for this systematic review was to give an updated assessment on the role of probiotics supplementation in patients with CRC compared to control and placebo and to assess the different primary and secondary outcomes of published RCT reports.

METHODS

This systematic review was prepared according to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols guidelines.[24] All the studies included in this review were RCTs of probiotic supplementation in patients with CRC. The protocol of this systematic review was registered in the PROSPERO database (registration no. CRD42020166865) and recently published.[25]

Search strategy

Five databases namely, Web of Science, PubMed, ProQuest, Wiley online library, and Scopus, were searched systematically and independently by 2 authors between January 17 and 20, 2020. The results of the search were scrutinized and filtered to identify RCTs published in English. The bibliography of each included study was assessed to identify additional RCTs missed during the initial search. The following search terms were selected according to the Population, Intervention, Comparison, Outcomes, and Study (PICOS) model[26]: (Probiotic OR Bifidobacterium OR Lactobacillus OR Saccharomyces OR Propionibacterium OR "Bacillus coagulans") AND (colorectal OR rectal OR colon OR colonic) AND (cancer OR carcinoma OR neopla* OR malignan* OR tumo*) AND ("randomised controlled trial" OR "clinical trial" OR "RCT" OR "intervention study").

Exclusion and inclusion criteria

The inclusion criteria for this systematic review were in line with the PICOS model and are summarized in Table 1. The study design had to be a randomized controlled trial, the participants and condition of interest include patients of any age who were diagnosed with either CRC, rectal cancer, or colon cancer, and the intervention of interest was probiotics either used individually or together with other probiotics or prebiotics such as inulin. The control group was either a placebo or a healthy individual of any age, or a baseline comparison was made between patients before the intervention. The exclusion criteria included non–English-language studies; reviews; animal or in vitro probiotic studies; noncolorectal, rectal, or colon cancer studies; and studies that did not assess the effect of probiotics in patients with CRC.

Outcomes of interest

The primary outcomes of interest extracted from the eligible RCTs included the effects of probiotics on the diversity of gut microbiota, the immunomodulatory effects of probiotics, the influence of probiotics on inflammatory biomarkers, and the effects of probiotics on the growth and development of colorectal tumors. The secondary outcomes comprised postoperative complications, hospital length of stay (LOS), quality of life (QOL), and death.

Data extraction

The studies were screened and selected independently by 2 authors (I.J.D. and M.A.A.) on the basis of the prespecified inclusion and exclusion criteria. The title and abstract of each RCT were first screened and then the full text was assessed to extract data from the most relevant studies that fit the inclusion criteria. The following data were extracted using a predesigned table: author(s) and publication year, participants' details, placebo details, type of probiotics, dosage and duration of intervention, primary outcomes and secondary outcomes (Table 2).[27–49] Discrepancies in study selections and data extraction were resolved by a third author (A.M.A. or S.H.) to avoid any bias in acquisition of relevant data.

Quality of evidence and risk-of-bias assessment

The methodological quality of selected individual RCTs was assessed independently by 2 authors (I.J.D. and M.A.A.) using the RoB 2.0 tool[50] according to the guidelines of the Cochrane Collaboration, using Review

Table 1 **PICOS criteria for inclusion of studies**

Parameter	Criteria
Participants/population	Patients of any age with CRC treated with probiotics, have undergone colorectal resection surgery
Intervention	Probiotics (eg, *Bifidobacterium*, *Lactobacillus*, *Saccharomyces*) in combination with other probiotics or with prebiotics such as oligosaccharides or inulin
Comparison	Placebo group, control group with CRC who did not receive treatment
Outcomes	Probiotic effect on modulation of human gut microbiota
	Modulation of immune system and inflammatory biomarkers relevant to CRC
	Reduction of postoperative complications
	Length of hospital stay
	Death
	Reduction in tumor size
Study design	Randomized controlled clinical trial

Manager software (RevMan, version 5.4; Cochrane Collaboration, Copenhagen, Denmark).[51] The risk-of-bias evaluation was carried out according to the following domains: allocation concealment, random sequence generation, blinding of participant, blinding of outcomes assessment, insufficient outcome results, selective reporting, and other sources of bias. Any conflicts in the risk of bias assessment were discussed and resolved by seeking additional opinion from a third author.

Using the PRISMA checklist[24] (Table S1 in the Supporting Information online), the quality of all included studies was assessed as was the absence of publication bias. The robustness of the findings and the quality of evidence of outcomes (primary and secondary) were rated using GRADEpro software.[52] Grading of Recommendations, Assessment, Development and Evaluations (GRADE) has 4levels of certainty categorized as high-quality, moderate, low or very low-quality evidence. The quality of evidence using the GRADE approach was assessed independently by 2 authors across each outcome on the basis of publication bias, risk of bias, imprecision, inconsistency, and indirectness of evidence (Table 3).[53]

RESULTS

Study selection

A total of 956 studies were obtained from a primary search of Web of Science, Scopus, ProQuest, the Wiley online library, and PubMed databases. After duplicates (n = 226) were removed, using EndNote software, 730 articles remained. Of these, 695 articles were excluded during the title- and abstract-screening step, because they were either animal studies, reviews, letters, conference papers, or not relevant to the scope of this review. The remaining 35 full-text articles were screened for eligibility and 12 studies were excluded. One was not an RCT,[54] another was a repeated work of an included study published in another journal,[55] and 10 other studies were excluded for different reasons, such as their outcomes did not meet the inclusion criteria,[56–60] the participants were healthy humans instead of patients with CRC,[61,62] the intervention was prebiotics alone without probiotics,[12] and the intervention used was not probiotics.[63,64] Finally, 23 RCTs met the inclusion criteria and were included in this systematic review. The flowchart of the literature screening process is shown in Figure 1.

Overview of selected studies

All 23 included studies were double- or single-blind RCTs. Altogether, there were a total of 2457 participants, with 1054 in the control/placebo group and 1403 participants in the intervention group (Table 2). The longest intervention was 4 years[33] and the shortest was 3 days.[38] The age range of participants in these RCTs ranged from 18 to 92 years. Tumor size differed among the participants. Of the 23 RCTs, 12[27,29–32,37,39,40,42,47–49] (52.2%) used a mixture of probiotics for the intervention, 7 studies[28,33,35,36,38,44,45] (30.4%) used synbiotics, 3[34,41,43] (13.0%) used single probiotics, and 1study[46] (4.3%) used kefir as the probiotics source. Species of *Bifidobacterium, Saccharomyces boulardii*, and *Lactobacillus* are the main probiotic microorganisms used in the manufacture of probiotic products such as yogurt, miso, and kefir. Placebo was used in 16 studies (69.6%) and 7 studies (30.4%) used a control healthy group as a comparator.

Risk-of-bias assessment

Collectively, there was a low risk of bias in the included RCTs. A computer-generated sequence was used for randomization of participants into intervention or control groups in all the studies. Treatment allocation concealment was done in all but 4 RCTs.[35,36,44,46] Blinding of participants and personnel risk was high in 3 studies.[33,35,36] Only 3 of the 23 RCTs[36,41,46] reported incomplete data. The blinding of outcome assessment risk was

Table 2 **Study characteristics and outcomes of 23 included RCTs**

Reference	Participants			Placebo/comparator				Stage of cancer	Age of participants (years)	Intervention	Dosage/ duration	Primary outcomes	Secondary outcomes
	No.	Mean age (years)	Sex, male/ female	Type of comparator	No.	Mean age (years)	Sex male/ female						
Consoli et al. (2016)[27]	15	51	5/10	Conventional treatment	18	51	10/8	1–4	17–83	Oral lyophilized yeast capsule with 100 mg (0.5×10^9 CFU of *Saccharomyces. boulardii)*	One capsule/ day for 7 days	Reduced expression of pro-inflammatory cytokines in probiotic group	Alleviation of post-operative complications
Flesch et al. (2017)[28]	49	64.5	18/31	96% maltodextrin 100% (6 g)	42	61.1	19/23	1–4	18– 89	*B. lactis* HN019, *Lactobacillus paracasei* LPC-, *L. rhamnosus, L. acidophilus* NCFM and fructo-oligo-saccharides, 6 g	Two sachets twice daily for 5 days before the surgical procedure and for 14 days after surgery	N/A	Decreased infectious and non-infectious postoperative complications after surgery Decreased SSI among treatment group
Franko et al. (2019)[29]	67	62.0	32/35	No placebo	68	68.1	37/31	1–4	20– 88	VSL#3; Alfasigma capsule containing 8 different strains of live LAB with 112.5 billion CFU/capsule: *B. infantis*, *L. plantarum, L. paracasei, B. longum, L. bulgaricus, L. acidophilus, B. breve*, and *Streptococcus thermophilus*	Twice daily postoperatively for $\leq$15 doses or until discharge	N/A	Less use of unplanned therapeutics in probiotic group Improved quality of life in probiotic group Fewer complications in probiotic group Lower re-admission rate in probiotic group
Gao et al. (2015)[30]	11	65.9	6/5	Encapsulated maltodextrin and tissue from healthy patients	22	69.7	12/10	1–3	40–75	Preparation containing live combined *L. acidophilus, B. longum,* and *Enterococcus faecalis* (1:1:1)	6.0×10^7 CFU/ g viable cells, 3 times/day, for 5 days	Probiotics modulate gut microflora structure	N/A
Gianotti et al. (2008)[31]	21	64.7	7/8	Maltodextrin only	10	63.3	7/3	1–4	18–54	1:1 mixture of *B. longum* (BB536) and *L.*	10^7 CFU (low dose), probiotics at	Positive colonization and adhesion of	Negative culture for

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Table 2 **Continued**

Reference	Participants			Placebo/comparator				Stage of cancer	Age of participants (years)	Intervention	Dosage/ duration	Primary outcomes	Secondary outcomes
	No.	Mean age (years)	Sex, male/ female	Type of comparator	No.	Mean age (years)	Sex male/ female						
										johnsonii in spray-dried form and blended with maltodextrin	10^9 CFU (high dose), twice daily for a total of 6 days	probiotic bacteria (*Lactobacillus*) to colonic mucosa Change in microbiota composition and in stool	*Clostridium perfringens* from colonic mucosa
Golkhalkhali et al. (2018)[32]	70	65	N/A	Biologically inactive placebo	70	66	N/A	N/A	18–89	MCP preparation containing 30 billion CFU and omega-3 fatty acid per sachet	30 billion CFU/ sachet, 1 sachet daily for 4 weeks	pPobiotic modulated the immune system by reducing pro-inflammatory cytokine IL-6 and TNF-α levels in treatment group	Improvement of quality of life in probiotic group
Ishikawa et al. (2005)[33]	301	54.5	242/59	Dietary instruction alone	97	55.5	83/14	Early of cancer	40– 65	Three regimens: A, (dietary instruction+ regular intake of wheat bran biscuits); B, dietary instruction + regular intake of *L. casei* preparation); C, (dietary instruction + regular intake of wheat bran biscuits + *L. casei* preparation)	Wheat bran (25 g/day), *L. casei* (10^{10} viable cells/1 g/ day), once daily for 4 years	Overall reduction of tumor development	N/A
Kakaei et al. (2019)[34]	50	50.08	29/21	Placebo	50	48.92	26/24	1–4	18–80	*Lactobacillus* probiotic capsule	1 probiotic capsule daily for23		Improvement in length of

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Reference	Participants			Placebo/comparator				Stage of cancer	Age of participants (years)	Intervention	Dosage/ duration	Primary outcomes	Secondary outcomes
	No.	Mean age (years)	Sex, male/ female	Type of comparator	No.	Mean age (years)	Sex male/ female						
											days after surgery and 7 days before surgery		hospital stay in probiotic group, though no significant
Komatsu et al. (2018)[35]	33	74.1	14/19	No placebo	39	69.6	23/16	1–4	30– 92	1 Bottle of Yakult Ace containing $\geq 3 \times 10^{10}$ living *L. casei* strain Shirota with 2.5 g galactoligosaccharides; 1 bottle of MILMIL-S, which contained $\geq 1 \times 10^{10}$ living *B. breve*	80 mL of Yakult Ace, 100 mL of MILMIL-S, and 2.5 g galacto-oligosaccharides, once daily for 7–11 days before surgery	No significant correlations between the ileal microbes and tumor stage. Negative correlation between tumor size and *Bifidobacterium*, intake of *Bifidobacterium* probiotic	Changes in ileal microbiota More colonization of symbiotic group with *L. casei*
Komatsu et al. (2016)[36]	168	66.7	92/76	No placebo	194	67.7	118/76	1–4	28–89	1 Bottle of Yakult Ace, which contained $\geq 3 \times 10^{10}$ living *L. casei* strain Shirota with 2.5 g galactoligosaccharides; 1 bottle of MILMIL-S, which contained $\geq 1 \times 10^{10}$ living *B. breve*	80 mL of Yakult Ace, 100 mL of MILMIL-S, and 2.5 g galacto-oligosaccharides, once daily for 7–11 days before surgery	Increased total bacterial numbers and dominant obligatory anaerobes in the probiotic group	SSI occurrence in control group more than in probiotic group
Kotzampassi et al. (2015)[37]	84	65.9	57/27	Powdered glucose polymer	80	66.4	58/22	1–4	18 –89	Mixed probiotics capsule (*L. acidophilus* +*L. plantarum* + *B. lactis* + *S. boulardii*)	One capsule, twice a day with 100 mL of drinking water twice a day for 14 days	Positive correlations between the gene expression of SOCS3 and the gene ex-pression	Shorter hospital LOS in probiotic group The overall complication rate

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Table 2 **Continued**

Reference	Participants			Placebo/comparator				Stage of cancer	Age of participants (years)	Intervention	Dosage/ duration	Primary outcomes	Secondary outcomes
	No.	Mean age (years)	Sex, male/ female	Type of comparator	No.	Mean age (years)	Sex male/ female						
												of TNF, as well as between the gene expression of SOCS3 and circulating IL-6 in the probiotic groups. More isolation of pathogenic microbes in control group than probiotic group	increased in placebo group and reduced in probiotic group
Krebs, (2016)[38]	38	64.5	24/14	No placebo	16	67.0	9/7	1–4	20– 78	Synbiotic 2000 FORTE 1011 sachet containing a mixture of 4 LAB+ 2.5 g of each of the 4 fermentable fibers	1 sachet twice daily for 3 days before the surgery	Higher concentrations of lactobacilli in patients who received synbiotics preoperatively. The difference was statistically significant for all 4 LABs.	Low complication rates in both groups
Lee et al. (2014)[39]	28	56.36	15/13	Placebo pills composed of ascorbic acid, magnesium stearate, and maltodextrin	32	56.03	20/12	2–3	20– 86	The probiotic preparation (Lacidofil) contained *L. rhamnosus* + *L. acidophilus* magnesium stearate, ascorbic acid and maltodextrin.	1 Tablet twice daily for 12 weeks	Significant decrease of irritable bowel syndrome in probiotic group compared to placebo group	Improved quality of life in probiotic group, though not statistically significant
Liu et al. (2013)[40]	75	66.06	38/37	Maltodextrin only	75	62.28	40/35	1–3	25–75	Encapsulated mixture of 3 probiotics bacteria, composed of *L. plantarum* +	1 capsule containing 2.6 $\times 10^{14}$ CFU/day for 16 days, 6 days	p38 MAPK expression was lower in probiotics group (1.5860.59)	Lower postoperative incidence of BT in probiotics group

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Table 2 **Continued**

Reference	Participants			Placebo/comparator				Stage of cancer	Age of participants (years)	Intervention	Dosage/ duration	Primary outcomes	Secondary outcomes
	No.	Mean age (years)	Sex, male/ female	Type of comparator	No.	Mean age (years)	Sex male/ female						
										L. acidophilus-11 + *B. longum*-88	before surgery and 10 days after surgery	than in control group (2.4960.58; $P = 0.001$).	The postoperative serum zonulin level decreased with probiotics. Postoperative infectious complications were alleviated by probiotics. Postoperative variables related to infection varied significantly between probiotics and control groups.
Mangell et al. (2012)[41]	32	74.0	16/16	Oatmeal-based drink without bacteria	32	70.2	20/12	1–4	64 –80	Oatmeal-based drink containing 10^9 CFU of *L. plantarum* Lp 299v/mL	100 mL Daily for 8 days before surgery and 5 days after surgery	N/A	Improved restriction of BT in probiotic group compared to placebo group Lower rate of postoperative complications in probiotic group, though no significant difference in the complication rate between the 2 groups

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Table 2 **Continued**

Reference	Participants			Placebo/comparator				Stage of cancer	Age of participants (years)	Intervention	Dosage/ duration	Primary outcomes	Secondary outcomes
	No.	Mean age (years)	Sex, male/ female	Type of comparator	No.	Mean age (years)	Sex male/ female						
Mego et al. (2015)[42]	23	62.0	14/9	Additives without bacteria: inulin (40%), maltodextrin (31.4%), magnesium stearate (3%), ascorbic acid (0.4%)	23	64.0	12/11	1–4	42– 81	Colon Dophilus probiotic formula capsule containing 10 lyophilized probiotic strains	1 capsule 3 times daily orally for 12 weeks	N/A	Patients in probiotic arm used less loperamide and diphenoxylate/ atropine compared to patients in placebo arm. Reduction of incidence and severity of adverse effects and postoperative complications
Mizuta et al. (2016)[43]	31	68.9	20/11	No placebo	29	71.2	15/14	1–4	20–85	Sachet containing 2 g of *B. longum* BB536 powder ($\sim 5 \times 10^{10}$ colony-forming units/2 g)	1 sachet daily for 7–14 days preoperatively and 14 days		
			postoperatively	Changes in fecal microbiota High-throughput sequencing analysis of the fecal microbiota at the phylum level indicated significant postoperative increases in the proportion of beneficial bacteria in probiotic group than placebo group.		The rates of postoperative infections higher in placebo group but did not differ significantly between the probiotic and control groups The hospital LOS was significantly shortened in the probiotic group						

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Table 2 **Continued**

Reference	Participants			Placebo/comparator				Stage of cancer	Age of participants (years)	Intervention	Dosage/ duration	Primary outcomes	Secondary outcomes
	No.	Mean age (years)	Sex, male/ female	Type of comparator	No.	Mean age (years)	Sex male/ female						
Roller et al. (2007)[44]	19	62.1	N/A	10 g Maltodextrin	15	60.1	N/A	1–4	19–75	Inulin enriched with oligofructose in combination with the PRO *L. rhamnosus* GG (LGG) + *B. lactis* Bb12 (Bb12)	1 capsule of bacteria and 10 g of prebiotics daily for 12 weeks	The percentage of neutrophils that produced ROS and the rate of productions in both study groups were not affected by the SYN intervention. The intake of SYN in both groups did not significantly alter the lytic behavior of NK cells. No significant difference in the production of TNF-α, IL-10, and IL-12 cytokines SYN significantly enhanced IFNγ production by PBMCs	N/A
Theodoropoulos et al. (2016)[45]	38	66.6	20/18	Four fibers and no LAB	37	69.0	23/14	1–4	18–80	FourLAB: *Pediococcus pentosaceus* + *Leuconostoc mesenteroides* + *Lactobacillus paracasei* + *L. plantarum*, and 2.5 g of each of the 4 fermentable fibers: β -glucan, inulin, pectin, and resistant starch	12 g in 250 mL of water once daily for 15 days	N/A	Improvement of functional bowel disorders diarrhea, constipation in probiotic group Improved gastrointestinal function–related quality of life in probiotic group

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Table 2 **Continued**

Reference	Participants			Placebo/comparator				Stage of cancer	Age of participants (years)	Intervention	Dosage/ duration	Primary outcomes	Secondary outcomes
	No.	Mean age (years)	Sex, male/ female	Type of comparator	No.	Mean age (years)	Sex male/ female						
Topuz et al. (2008)[46]	163	51.8	12/151	Oral lavage with 0.09% NaCl	20	58.1	12/8	2–3	19–75	Kefir	250 mL of kefir twice daily after meals on the first 5 days of chemotherapy	Serum pro-inflammatory cytokine levels were measured in both groups at baseline and after the third and sixth cycles of CT, and there was no significant difference between the 2 groups ($P > 0.05$).	Kefir inhibits the growth of *S. epidermidis* at 8 mm at 12 h, 18 h, and 24 h intervals under in vitro conditions Increased incidence of mucositis in placebo group compared with kefir group
Yang et al. (2016)[47]	30	63.90	15/15	Placebo powder containing maltodextrin and sucrose without any viable probiotics	30	62.17	12/18	1–3	25–80	Combined probiotics (live combined *B., L.* + *Enterococcus, B. longum* +*L. acidophilus*)	Once daily for 5 days before and 7 days postoperatively	N/A	Significant improvement of postoperative short-term outcomes in the probiotics group than the placebo group Reduction of infectious complications such as bacteremia, wound infection, and diarrhea in probiotic group

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Table 2 **Continued**

Reference	Participants			Placebo/comparator				Stage of cancer	Age of participants (years)	Intervention	Dosage/ duration	Primary outcomes	Secondary outcomes
	No.	Mean age (years)	Sex, male/ female	Type of comparator	No.	Mean age (years)	Sex male/ female						
Zaharuddin et al. (2019)[48]	27	67.3	19/8	Placebo	25	66.6	15/10	1–4	23–85	Probiotics contained 30 billion CFU of 6 viable *Lactobacillus* and *Bifidobacteria* strains	1 sachet taken orally twice daily for 6 months	Reduced production of serum pro-inflammatory cytokines (IL-12, IL-10, TNF-α, IL-6, IL-17A, IL-17C, and IL-22) in probiotic group	N/A
Zhang et al. (2012)[49]	30	67.5	10/20	Maltodextrin capsules	30	61.5	14/16	1–4	45–82	3 oral bifid triple viable capsules, each of which contained 0.21 g (108 CFU/g) of *B. longum*, *L. acidophilus*, and *E. faecalis*	3 capsules, 3 times a day for 3 days	Increased count of fecal bacterial colonies (*Escherichia coli, B. longum*) in placebo group compared with probiotic group. Serum IgM and IgA levels did not differ significantly between groups in the preoperative or postoperative periods	Reduced postoperative complications in probiotic group compared with placebo group Restricted BT in probiotic group compared with placebo group

Abbreviations: CT, chemotherapy; LAB, lactic acid bacteria; PBMC, peripheral blood mononuclear cell; N/A, not applicable, NK, natural killer; ROS, reactive oxygen species; SOSC3, suppressor of cytokine signaling 3; SYN, synbiotic.

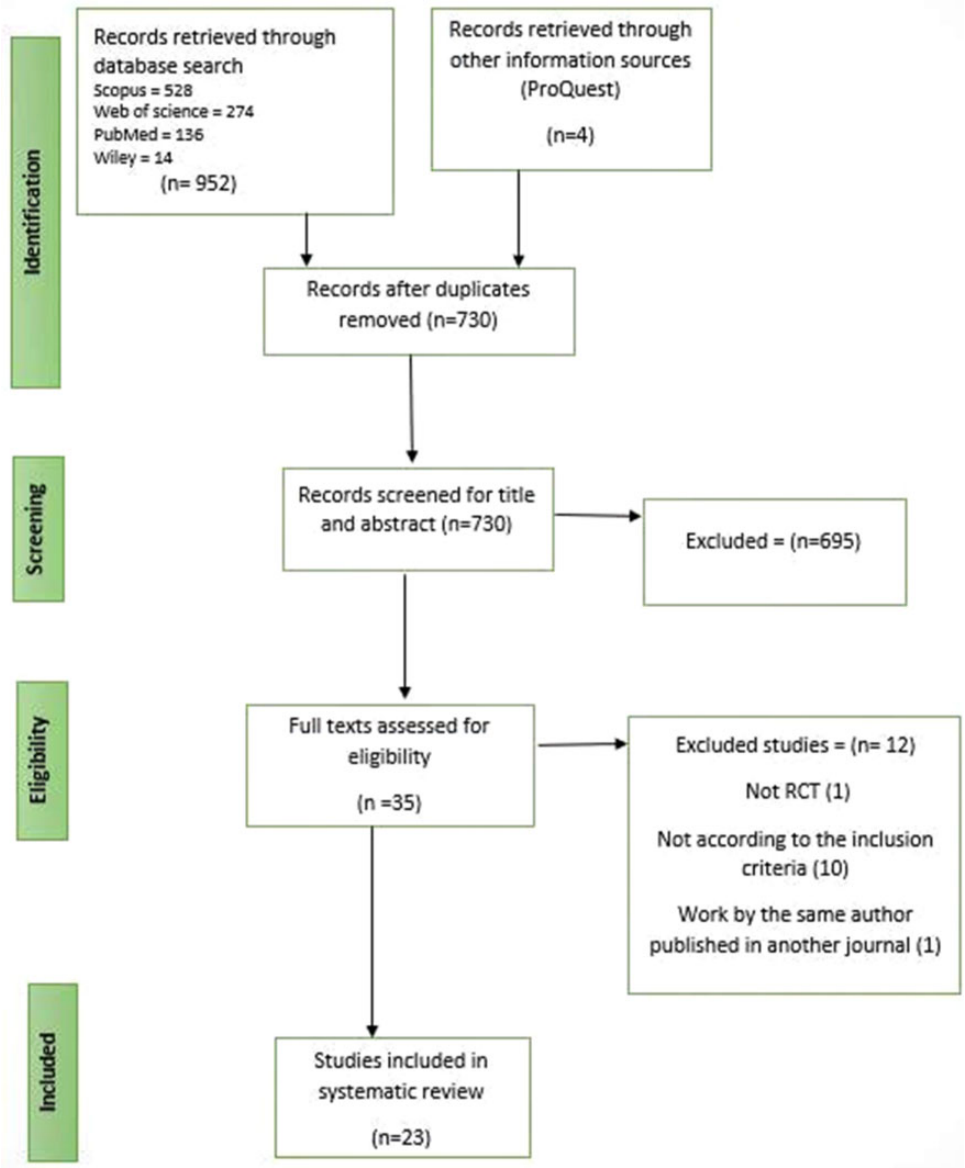

Figure 1 **PRISMA flowchart showing the literature screening and study selection process**

unclear in 14 studies[27–30,34,40–47,49] and was high in 3 studies.[33,35,36] Three studies[29,37,42] were ended prematurely, and in 1 RCT,[30] additional healthy patients were included in the trial when the researchers realized the initial sample size was too small, leading to other kind of bias. The summary results of the risk-of-bias assessment are shown in Figure 2.

Quality of the evidence

The GRADE analysis (Table 3) showed that the certainty of 4 outcomes was high because there was directness of evidence, precision, consistency, and no serious risk of bias across the studies. These 4 outcomes were modulation of the immune system and inflammatory biomarkers, hospital LOS, improved quality of patient's life, and colorectal tumor growth. The certainty of the other 3 outcomes, namely, modulation of gut microbiota, postoperative complications, and death, was moderate, due to a serious certainty assessment in the risk-of-bias domain and indirectness of evidence.

Outcomes

Modulation of gut microbiota. The gut microbiota comprise diverse numbers and groups of microorganisms, which makes it one of the most complex parts of the human body. The status of the gut microbiota affects overall human health as well as gastrointestinal functions. Different situations can affect the status and health of

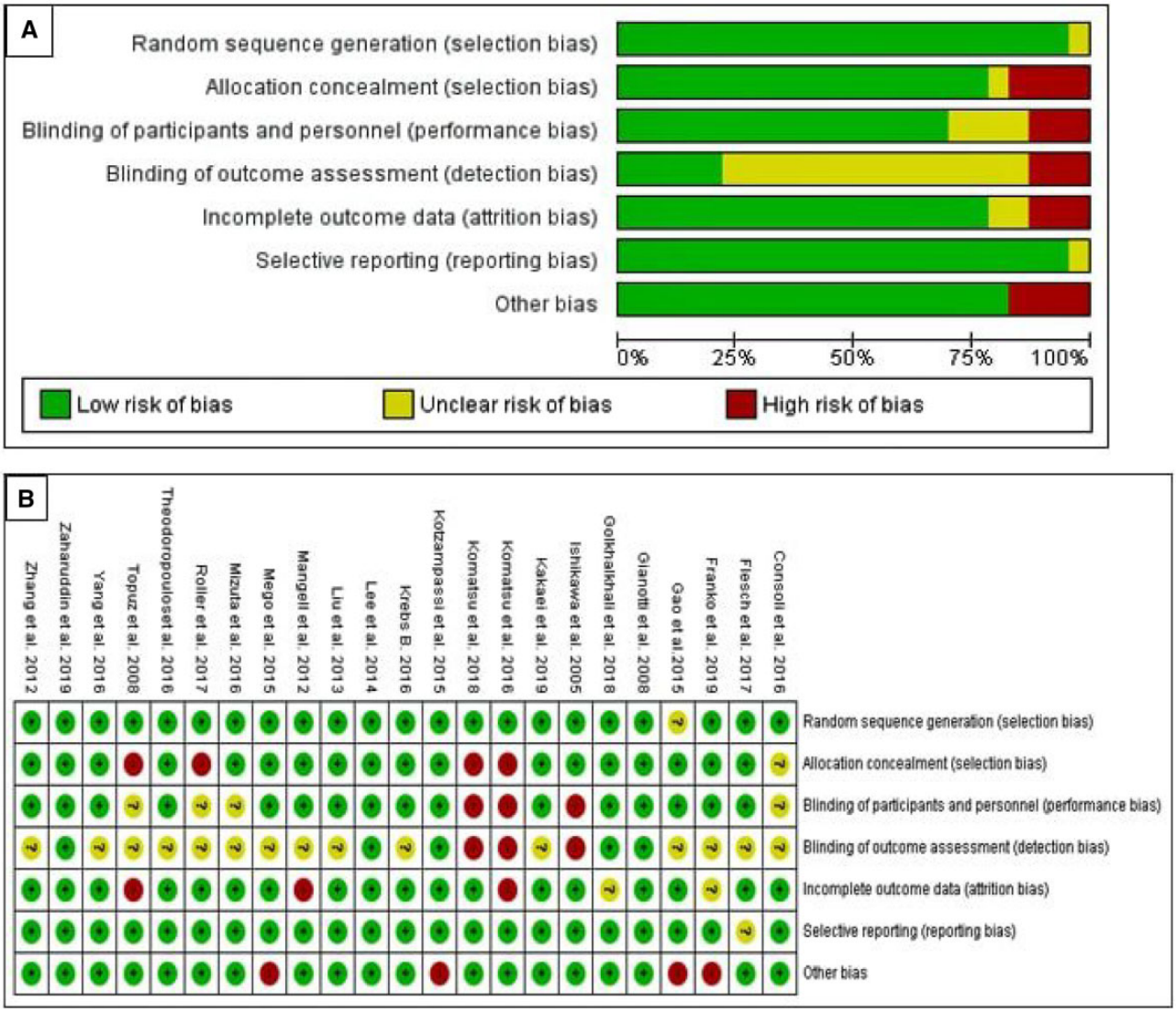

Figure 2 **(A) Risk-of-bias assessment graph according to RoB 2.0 tool; (B) risk-of-bias summary of 23 included RCTs, using RevMan, version 5.4**

the gut microbiota positively or negatively, such as dietary habits of an individual, antibiotics use, stress levels, smoking, and hereditary factors.[65] Dysbiosis of the gut microbiota affects the gut health negatively and can lead to tumorigenesis. Few studies reported the gut microbiota of patients with CRC is different from that of healthy individuals, and consumption of probiotics was seen to affect the gut microbiota composition positively, leading to a more balanced microbiota environment and decrease in pathogenic microbes.[66]

Six RCTs evaluated the effects of probiotic administration on the gut microbiota.[30,31,35,36,43,49] One study assessed the differences of the gut microbiota of 2 groups of patients with CRC and 1 group of healthy individuals. The first CRC patient group received an intervention of 6.0×10^7 CFU/g viable cells of combined *Enterococcus faecalis, L. acidophilus,* and *B. longum* (1:1:1), 3 times/day for 5 days. The other CRC patient group received placebo, which was maltodextrin, and the healthy individuals received nothing. Colonic mucosal biopsy specimens were collected from all the participants and tested. A comparison of the gut microflora diversity among the groups showed the diversity of mucosal microflora was decreased in patients with CRC, but after oral administration of probiotics, the diversity and richness increased. A comparison of the gut microbiota structure at different levels indicated there no major variations in classification at the phylum level among the 3 groups. Pyrosequencing results showed a substantial reduction in *Fusobacterium, Peptostreptococcus,* and *Comamonas* populations and expansion of Proteobacteria and *Enterococcus* in the mucosa-adherent microbiota in patients treated with probiotics.

To evaluate the variations between microbiota in patients with CRC and that in healthy people, a

Table 3 **GRADE assessment of the quality of evidence**

Outcome	Certainty assessment*						No. of participants		Certainty
	No. of studies	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Probiotic/synbiotic	Placebo or control group	
Modulation of gut microbiota	6	Serious [a]	Not serious	Not serious	Not serious	None	360	370	⊕⊕⊕◯ Moderate
Modulation of immune system and inflammatory biomarkers	4	Not serious	Not serious	Not serious	Not serious	None	131	128	⊕⊕⊕⊕ High
Postoperative complications	18	Serious [b]	Not serious	Not serious	Not serious	None	878	874	⊕⊕⊕◯ Moderate
Length of hospital stay	7	Not serious	Not serious	Not serious	Not serious	None	289	269	⊕⊕⊕⊕ High
Quality of life improvement	4	Not serious	Not serious	Not serious	Not serious	None	136	139	⊕⊕⊕⊕ High
Colorectal tumor growth and tumor stages development	2	Not serious	Not serious	Not serious	Not serious	None	335	140	⊕⊕⊕⊕ High
Death	2	Not serious	Not serious	Serious [c]	Not serious	None	117	118	⊕⊕⊕◯ Moderate

*All studies were randomized trials.
[a]Two studies have a high risk of bias based on allocation concealment, blinding of participants, and outcome assessment.
[b]Four studies have a high risk of bias in selective outcome reporting and three studies were prematurely ended.
[c]One study reported mortality rates not specifically for colorectal cancer patients but also for other cancer patients included in the trial
GRADE Working Group grades of evidence
High quality: The actual impact is close to that of the impact estimate.
Moderate quality: There is reasonably confidence in the estimate of the impact: it is possible that the true effect is similar to the estimate of the effect, but there is a chance that it is significantly different.
Low quality: Faith in the estimate of the effect is limited: the true effect could vary significantly from the estimate of the effect.
Very poor quality: There is little faith in the effect estimate: it is possible that the true effect will vary considerably from the effect estimate.

taxonomy-based comparison was also conducted. Less than 0.1% of the total bacteria in the mucosal tissue of healthy participants was made up of *Fusobacterium*, whereas it was the most abundant genus in the mucosa of patients with CRC (10.08% vs 0.01% in the healthy group; $P = 0.03$). However, the probiotics treatment resulted in a significant decrease in *Fusobacterium* relative abundance by ~6-fold in the probiotic group (1.91%; $P = 0.03$).[30]

The administration of probiotics (mixture of equal ratio of *B. longum* (BB536) and *L. johnsonii* (La1) mixed with maltodextrin to patients with CRC who underwent colorectal surgery influenced the adherence of *Lactobacillus* to colonic mucosa or stool colonization.[31] In this study, the patients with CRC were split into 3 groups: the high dose (10^9 CFU), low dose (10^7 CFU), and placebo groups. On the fourth day of probiotics administration, the adherence of La1 to the colonic mucosa or colonization of the stool was 60% (n = 6 of 10) in the group that received the high dose, 27.2% (n = 3 of 11) in the l group that received low dose, and 0% in the placebo group (n = 0 of 10) (placebo vs high-dose group, $P = 0.02$,). There was a significantly higher count of Lactobacilli cultured in stool samples in the group receiving the higher dose than in the placebo or low-dose groups ($P = 0.04$). Two studies[31,35] analyzed the change in microbiota composition, focusing particularly in stool count for Enterococci and Enterobacteriaceae members. There was a lower count of Enterobacteriaceae in high-dose stool samples than in the low-dose or placebo-treated groups (placebo vs high-dose group, $P = 0.07$). For the Enterococci, the same pattern was also recorded. The colonic mucosa adherence levels for Enterobacteriaceae was 30% in the high-dose group, 82% in the low-dose group, and 70% in the placebo group (high-dose vs low-dose, $P = 0.03$). In the Gianotti et al[31] study, *Clostridium perfringens* cultures were negative in stools and mucosa in all 3 groups. *Bifidobacterium longum* BB536 strain was not found at any point in the.[31]

In another study in which the intervention as 1 bottle of Yakult Ace, which incorporated 2.5 g of galactooligosaccharides and 3×10^{10} living *L. casei* strain Shirota and 1 bottle of MIL-MIL-S, which constituted $\geq 1 \times 10^{10}$ living *B. breve*, the Enterobacteriaceae count was also significantly higher in the control group than in the synbiotic group (± SD) (8.0 ± 1.2 vs 6.7 ± 1.2). In addition, the assessment of changes in the ileal microbiota showed that total amount of bacteria in the ileal mucus was$10^{8.5}$ cells/g in the group that received synbiotics and $10^{8.4}$ cells/g in the comparator group. Numbers of *Clostridium*, *B. fragilis*, and *Bacteroides* (obligate anaerobes) were predominant over facultative anaerobes, such as *Lactobacillus* spp.[35] Another RCT that used the same probiotic intervention assessed the effect of synbiotics on changes in fecal bacteria after surgery and found that the probiotic suppressed the growth of potentially pathogenic species, such as *C. difficile* while increasing *Bifidobacterium* and *L. casei* growth.[36] These findings suggest the influence of ingestion of synbiotics on the gut microbiota is mainly in the proximal colon connected with the terminal ileum, where fermentation by human pathogens is promoted in the delayed fecal flow. Other studies assessed changes in fecal microbiota by high-throughput sequencing analysis of the fecal microbiota at the phylum level, which indicated significant postoperative increases in the percentage of Bacteroidetes and Proteobacteria and significant decreases in those of Firmicutes and unclassified bacterial strains in the control group, although the percentage of Actinobacteria increased and that of Firmicutes significantly decreased in the probiotic group. The percentages of these bacterial strains revealed no significant difference in the various groups before or after surgery[43] or through fecal assays to determine the *Bifidobacterium*-to-*Escherichia* ratio, present postoperatively and preoperatively in placebo group ($P = 0.05$). Counts of *Bifidobacterium* increased significantly, whereas amount of *Escherichia* decreased significantly on days 3–5 postoperatively ($P = 0.05$), in addition to reversing the *Bifidobacterium*-to-*Escherichia* ratio for 3–5 days postoperatively in the probiotic group.[49] These studies showed that ingestion of probiotics by patients with CRC led to a decrease in population of pathogenic and inflammation-inducing microorganisms while causing an increase in the beneficial microorganism population, which promotes better gut homeostasis and production of SCFAs, which provide energy for the colon cells. Regular consumption of probiotic foods or nutraceuticals is encouraged not just for those with CRC but for all people, because the intake of probiotics promotes a healthy intestinal environment and prevents the overgrowth of pathogenic microorganisms that may lead to dysbiosis of the gut microbiome.

Modulation of the immune system and inflammatory biomarkers

Probiotics promote the regulation of the immune system, and continuous supplementation with probiotics helps modulate the intestinal microbiota and reduce the overexpression of pro-inflammatory cytokines.[67] In an RCT by Consoli et al,[27] the probiotic group received a lyophilized capsule of yeast with 100 mg (0.5×10^9 CFU/g) of *Saccharomyces boulardii* orally. Mucosal samples from patients' tumor obtained during surgery showed that the mucosal cytokine mRNA expression

levels of IL-23A, IL-1β, and IL-10 were significantly lower ($P = 0.03$, $P = 0.001$, and $P = 0.04$, respectively) in the probiotic group compared to control group. The mRNA expression of other cytokines (IL-17A, INF-γ, TNF-α, and IL-12B) was not significantly different between the 2 groups ($P > 0.05$), which could be attributed to the short duration of the intervention, which was not sufficient to influence the expression level of the cytokines. In addition, when probiotics were used in combination with omega-3 fatty acid, they modulated the inflammatory markers of patients with cancer who were undergoing chemotherapy. The use of a microbial preparation containing a mixture of probiotic bacteria (*L. lactis*, *Bifidobacterium infantis*, *L. acidophilus*, *B. longum, L. casei*, and *B. bifidum* 30 billion CFU/sachet) combined with omega-3 fatty acid significantly decreased the amount of IL-6 ($P = 0.002$) in the intervention group, but the IL-6 level increased in the group that received placebo. In the group that received probiotics, the levels of TNF-α were unchanged, whereas there was a significant increase in IL-6 and TNF-α levels in the group that received placebo.[32] These findings indicate a lowered rate of inflammation in the intervention group and higher rate of inflammation in placebo group. In a study by Roller et al,[44] in which the effects of daily administration of synbiotic composed of inulin-enriched oligofructose mixed with *B. lactis* Bb12 and *L. rhamnosus* GG was given to patients with CRC who had no other treatment apart from surgery, secretion of IL-2 was not influenced by synbiotic treatment. There was no significant difference in the levels of cytokines such as IL-12, IL-10, and TNF-α as a result of the treatment. However, in the treatment group, administration of synbiotics significantly increased the IFN-γ–producing capacity of peripheral blood mononuclear cells at 6 weeks and 12 weeks. The percentage of phagocytic, active neutrophils and monocytes and their phagocytic intensity, as well as the percentage of neutrophils that generated reactive oxygen species and lytic activity of natural killer cells, were not modulated or significantly affected by the synbiotic intervention.

In another study, probiotics administration (30 billion CFU from 6 viable strains of *Lactobacillus* and *Bifidobacterium*, including *B. longum*, *L. lactis*, *L. acidophilus*, *L. casei*, *B. bifidum*, and *B. infantis*) resulted in significantly lower serum levels of IL-12 ($P = 0.005$), IL-22 ($P = 0.018$), IL-17A ($P = 0.00$), TNF-α ($P = 0.002$), IL-17C ($P = 0.018$), and IL-10 ($P = 0.028$) in patients as compared to their baseline levels. However, patients who consumed placebo had significantly increased levels of serum cytokines IL-12 ($P = 0.028$), IL-22 ($P = 0.018$), IL-17C ($P = 0.028$), and TNF-α ($P = 0.005$) as compared to baseline. Their IL-17A and IL-10 levels were also marginally raised, but these increments were insignificant. Furthermore, the level of IL-6 was significantly lowered in both groups. At 6 months post-intervention, the IL-6 level was significantly reduced from ($\pm$ SEM) 3.88 ± 3.41 pg/mL to 1.44 ± 1.39 pg/mL in patients who received probiotics. The levels of IL-6 were also significantly reduced from ($\pm$ SEM) 4.25 ± 4.047 pg/mL to 0.91 ± 0.49 pg/mL among patients who received placebo.[48] On the other hand, there was no significance difference in the levels of IFN-γ pre- and postintervention in patients with CRC who obtained received either a placebo or probiotics. This impressive modulation of the immune system in the treatment group is evidence that longer and regular consumption of probiotics provides beneficial results for patients with CRC who are undergoing surgery and chemotherapy. The immunomodulatory effect of probiotics varies according to the strain, the use of >1 strain of probiotic, or addition of prebiotics that influence cytokine expression profile and the regulatory of T-cell response.

Reduction of postoperative complications

Patients with CRC undergo surgery intervention at some point during the course of the diseases. Although the surgical procedures and perioperative treatment have been remarkably improved, postoperative infection remains a major complication that prolongs patient hospitalization and increases costs, particularly after colorectal surgery. Surgical stress and the preparation of patients before the operation, such as mechanical bowel preparation, can worsen the gut-barrier integrity, restrict immune function, provoke systemic inflammation, upset the intestinal microbiota balance due to antibiotic therapy, and thus lead to postoperative infections.[68]

For surgical site infection (SSI), 4 RCTs[28,34,36,37] reported infections of surgical wounds and they all reported that the SSI incidence was greater in the control group than in the probiotic or symbiotic groups. In a study by Flesch et al,[28] only 1 patient in the symbiotics group who received *L. acidophilus* NCFM, *B. lactis* HN019, *L. rhamnous, L. paracasei*, and fructo-oligosaccharides had a surgical wound infection, whereas 9 patients had surgical wound infection in the control group ($P = 0.002$).[28] Kakaei et al[34] reported that cutaneous and subcutaneous tissues infection developed at the surgery site in 3 patients (6%) in the probiotic group and 5 patients (10%) in control group; the difference, however, was not significant ($P = 0.46$). In another RCT, 29 patients (17.3%) in the group that received synbiotics had SSI and SSI developed in 44 patients (22.7%) in the control group within 30 days of surgery, but the disparity was not statistically significant ($P = 0.20$).[36] Another study showed that 16 patients in the control group and 6 patients in the probiotic group (*L. acidophilus, B. lactis, S. boulardii, L. plantarum*) had

SSI ($P = 0.020$).[37] Taken together, these findings indicate that for patients with CRC scheduled for surgery, the administration of probiotics, especially species of *Lactobacillus* and *Bifidobacterium*, at least 7 days before and after the surgical procedure helps reduce the severity and incidence of SSI. Administration of probiotic before the surgical procedure ensures colonization of the gut with beneficial microorganisms that reduce the risk of an infection after invasive colorectal resection.

Regarding noninfectious complications such as diarrhea, intestinal obstruction, constipation, urinary tract infections, anastomotic leakage, patients requiring reoperation and readmission, pneumonia, bacteremia, and bloating, 14 studies[27–29,34,36–38,40–43,45,47,49] reported that patients exhibited single or multiple noninfectious postoperative complications of some sort, but across board, it was recorded that probiotic or synbiotic administration, containing mostly *Bifidobacterium*, *Lactobacillus*, and *Enterococcus* strains, reduced the severity of these complications. Twelve of these studies[27–29,34,36–38,41,43,45,47,49] reported no statistical difference between the treatment group and control/placebo group in the noninfectious complications, and 2 studies[40,42] reported a significant difference in the noninfectious complications among the treatment and control groups. Two other studies[34,41] also reported death due to the postoperative complications. The duration and dosage of the intervention used in these studies played a key role in the level of noninfectious complications that patients experienced. Higher doses ($\geq 2.6 \times 10^{14}$ CFU/day) and longer duration (1–6 months) resulted in a significant decline in the occurrence of noninfectious complications in the group that received the intervention as compared to the control group.

Three studies reported restriction of bacterial translocation (BT) due to probiotic intervention. An RCT there were fewer BT occurrences in the probiotics group, which received an encapsulated mixture of 3 probiotics composed of *L. plantarum*, *L. acidophilus*-11, and *B. longum*-88. The findings suggest that 28% of patients (n = 21 of 75) in the control group and 13.3% of patients (n = 10 of 75) in the probiotics group experienced BT. After treatment, the probiotic group had a significantly lower occurrence of BT than did the control group ($P = 0.027$).[40] Pretreatment with a supplement containing *L. plantarum* (Lp 299v) in another study did not prevent BT to the mesenteric lymph nodes. In total, 22 lymph nodes in the probiotic group and 24 lymph nodes in the placebo were tested for the presence of bacterial DNA. Bacterial translocation was reported in 9 lymph nodes, of which 6 were from the probiotic group and 3 were from the placebo ($P = 0.374$).[41] The insignificant difference may be as a result of the low dose (100 mL of an oatmeal-based drink with 10^9 CFU/mL Lp 299v), short time of pretreatment, and because only 1 probiotic strain was used in the study. Zhang et al[49] reported that BT restriction was identified in patients receiving 3 oral bifid triple viable capsules containing *L. acidophilus, E. faecalis,* and *B. longum* (0.21 g, 10^8 CFU/g each). The rate of *Escherichia coli* detection was rare in both groups on the third preoperative day and third postoperative day. The rate of BT in the control group was significantly higher than in the probiotic group (26.7% vs 3.3%; $P = 0.026$). levels of D-lactic acids and endotoxins derived from gut microbes also stayed relatively low, suggesting that BT from the intestinal walls via the mucosal barrier may have been limited by the treatment. Mucositis, which refers to the inflammation and painful swelling of the mucous membranes lining the digestive tract, is usually a side effect of receiving 5-fluorouracil chemotherapy. Mucositis was reduced with the administration of 250 mL of kefir after meals 2 times a day, though when compared between the kefir group and placebo group for mucositis development during chemotherapy, no statistical significance was detected ($P > 0.05$).[46] This might have been a result of a short duration of intervention administration and because the patients were undergoing chemotherapy as the kefir was administered; perhaps, the treatment should have continued even after chemotherapy for better results.

Postoperative complications, infectious or noninfectious, are of serious concern in the management of CRC, especially in patients who need colorectal resection. It was observed from the primary studies that there is a better outcome for postoperative complications when probiotic/synbiotic treatment is started ≥ 1 week preoperatively and continued postoperatively, because is a good strategy to minimize the incidence and severity of all types of postoperative complications. Probiotics and synbiotics can also be included in the bowel preparation regimen to boost gut-microbiota modulation and prevent overgrowth of pathogenic microorganisms.

Length of hospital stay

The effect of probiotic administration on the duration of hospitalization of patients with CRC undergoing surgery was evaluated in 7 RCTs. Among 33 patients who underwent colonic resections, the medial hospital LOS was 10 days in the probiotic group that received a lyophilized 100 mg yeast capsule of 0.5×10^9 CFU orally once a day; and 11 days in the control group. There was no significant difference ($P > 0.05$) between the groups.[27] In another study, 91 patients with CRC who underwent colorectal resection surgery were administered probiotics (a mixture of *L. paracasei, B. lactis*, *L.*

Table 4 **Ongoing clinical trials of probiotics in patients with CRC registered in ClinicalTrials.gov**

ClinicalTrial.gov identifier	Study title	Intervention	Control/placebo	Status	Start date–estimated completion date	Location
NCT03742596	Effect of probiotics supplementation on the side effects of radiation therapy on the immune system among colorectal cancer patients	Probiotic formula capsule containing (1×10^{10} CFU/g of *Lactobacillus* (*reuteri*, *rhamnosus*, *gasseri*, *acidophilus*, *paracasei*, *casei and plantarum; and Bifidobacterium lactis and B. longum*) and Bifidobacteria (*B. breve, bifidum, infantis*)	Normal treatment without any probiotic	Recruiting	November 7, 2018–December 30, 2022	King Hussein Cancer Center, Amman, Jordan
NCT02751736	The effect of probiotics from kimchi (CJLP243) on bowel function restoration after ileostomy closure in patients with rectal cancer: a pilot randomized controlled trial	2 g sachet (CJLP 243) containing 10 billion lactic acid bacteria (*L. plantarum* CJLP243), maltodextrin, glucose (anhydrous)	2 g sachet (near identically appearing placebo containing maltodextrin, glucose [anhydrous]) once daily for 3 weeks	Unknown	April 2016–December 2017	Seoul National University Bundang Hospital
NCT04021589	A study of chemotherapy with WeiLeShu versus chemotherapy alone in patients with metastatic colorectal cancer	WeiLeShu and chemotherapy	Chemotherapy alone	Recruiting	July 11, 2020–July 11, 2022	First affiliated hospital, Zhejiang University, Hangzhou, Zhejiang, China

(continued)

Table 4 **Continued**

ClinicalTrial.gov identifier	Study title	Intervention	Control/placebo	Status	Start date–estimated completion date	Location
NCT04131803	Efficacy and safety of probiotics combined with standard chemotherapy plus targeted therapy in patients with metastatic colorectal cancer: a prospective, open-label randomized, multi-center clinical trial	Bifico combined with chemotherapy plus targeted therapy	Chemotherapy plus targeted therapy	Not recruiting yet	July 25, 2020–November 5, 2023	Second Affiliated Hospital, School of Medicine, Zhejiang University
NCT03705442	Probiotics as adjuvant therapy in the treatment of metastatic colorectal cancer: randomized double-blind placebo-controlled trial	Dietary supplement: OmniBiotic 10	Placebo	Unknown status	February 9, 2018–February 9, 2020	Department of Radiotherapy and Oncology, Rijeka, Primorsko-goranska, Croatia
NCT00197873	Randomized, double blind, placebo controlled, cross-over phase II study on the effects of *L. rhamnosus* GG supplementation in patients on first-line XELOXA treatment for metastatic colorectal cancer	Dietary supplement: *L. rhamnosus*				
supplementation	Placebo	Unknown status	September 2005–	Department of Oncology,		

(continued)

Table 4 **Continued**

ClinicalTrial.gov identifier	Study title	Intervention	Control/placebo	Status	Start date–estimated completion date	Location
			December 2018	Helsinki, Finland		
NCT01579591	A phase III, randomized, double-blind placebo controlled study of the probiotic preparation VSL#3 vs placebo in increasing the pathological major response rate in patients receiving concurrent chemotherapy and pelvic radiation therapy	#VSL3 probiotic	Placebo	Unknown status	March 2012–March 2013	Catholic University of Sacred Heart-Rome, Rome, Italy

rhamnosus, L. acidophilus, and 6 g of fructo-oligosaccharides) for 5 days before surgery and for 14 days after colorectal resection. The findings also showed no significant difference in hospital LOS.[28] Another study reported a similar outcome with postoperative medial hospitalization time being (± SD) 15.00 ± 4.31 days for the placebo group and 15.86 ± 4.92 days for the probiotic group after receiving a mixture of probiotics (*Bifidobacterium*, *Lactobacillus*, and *Enterococcus* strains) for 5 days before and 7 days after surgery.[47] Kakaei et al[34] reported the duration of hospitalization for patients who received probiotics (1 capsule contained *Streptococcus thermophilus*, *B. longum* [1.5×10^9 CFU], *B. breve* [1.75×10^9 CFU], *L. acidophilus* [1.75×10^9 CFU], *L. plantarum* [0.5×10^9 CFU], and *L. casei* [1.75×10^9CFU]) was (± SD) 5.96 ± 2.53 days and for the control group was 6.10 ± 2.44 days; however, the statistical analysis showed no significant difference ($P = 0.30$).

Another RCT used a 4-probiotic regimen consisting of 0.5×10^9 CFU of *L. plantarum;* 1.5×10^9 CFU *B. lactis* BB-12; $1.75 \times 1.5 \times 10^9$ CFU of *S. boulardii*; and 1.75×10^9 CFU of *L. acidophilus* LA-5 per capsule as an intervention for 14 days. The findings showed that there was no great difference between the hospital LOS in the probiotic and placebo groups; the median hospital LOS in probiotic group was only 2 days less than that of the placebo group.[37] Furthermore, a study compared hospitalization time among 3 groups, where group A was given a mixture of synbiotics contains *Pediococcus pentosaceus, L. plantarum*, *Leuconostoc mesenteroides*, and *Lactobacillus paracasei* subsp. *paracase*i plus 2.5 g of inulin, pectin, resistant starch, and β glucan; group B was given prebiotics alone, which was just 2.5 g of inulin, pectin, resistant starch, and β glucan; and group C was the control. The median hospitalization duration was 10.16 days in group A, 10.5 days in group B, and 11.3 days in group C. In the control group, the hospitalization LOS but not statistically so ($P = 0.512$).[38] Only 1 study reported a significant ($P = 0.03$) decrease in the length of hospitalization in the treatment group (± SD) (39.6 ± 15.6 days) compared to the control group (21.6 ± 11.7 days). The patients in this study received an intervention containing 2 g of *B. longum* BB536 powder (5×10^{10} CFU) daily for 7–14 days preoperatively and 14 days after colorectal surgery.[43] Most published studies reported a nonsignificant difference in the length of hospitalization between probiotics and control/placebo groups; this was most likely due to variations in duration, strains, and dosage of probiotic administration. Generally, probiotics administration reduces postoperative complications and improves the outcomes of surgery, thus decreasing the need for patients to remain hospitalized for a longer time.[69]

Colorectal tumor growth and tumor stages

Probiotics ingestion combats CRC by different mechanisms, of which 1 is apoptosis, a protective mechanism against uncontrolled cell growth that leads SCFA production that, in turn, triggers apoptosis of cancer cells by deregulating pro-apoptotic pathways such as the NF-κB pathway.[70]

The reduction of colorectal tumor development due to *Lactobacillus caesi* plus wheat bran administration was reported by Ishikawa et al.[33] In their study, the intervention given over 4 years significantly suppressed the growth of tumor with moderate and severe atypia. The relationship between ileal microbiota and tumor stage was tested in another RCT that showed no significant correlation between the tumor stage and the number of ileal microbes. In addition, there was no significant correlation between the number of microbes present in the ileal microbiota and the size of the tumor, with the exception of a negative correlation (Pearson $r = -0.324$; $P = 0.015$) between *Bifidobacterium* number and the size of the tumor.[35]

It is postulated that intake of probiotics and prebiotics can aid in lowering the development and progression of colorectal tumors. However, an extended period of probiotic administration and increased dosage provide more benefit of tumor suppression.

Improvement in QOL for patients with CRC

Most published RCT reports showed a distinct improvement in the QOL of patients with CRC in the treatment group compared to the control group. To validate the data, a general 7-item questionnaire (Functional Assessment of Cancer Therapy (FACT)-G7 was used to assess the general QOL and well-being of participants before surgery and during the first postoperative visit. A deterioration in the measured QOL parameters (namely, diet sensitivity, bloating, and dehydration) was observed in the control group, which was assessed during the first postoperative clinic visit as compared to before surgery (± SD) (21.6 ± 3.9 vs 18.0 ± 6.3 points, respectively; $P = 0.019$). On the other hand, patients in the probiotic group who received 15 doses of a commercially available probiotic (VSL #3 capsule, Alfasigma USA Inc) did not record a deterioration in the QOL (16.3 ± 5.1 points vs 17.1 ± 5.0 points; $P = 0.327$).[29]

In another study, the QOL of patients with CRC was assessed according to the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30, which consists of 9 multi-item scales: five functional scales (ie, cognitive, social, physical, role, and emotional), 3 symptoms scales (ie, pain,

nausea and vomiting, and fatigue), and a scale of global health and QOL. The global health status recorded at the beginning of the study was (± SD) 53.70 ± 2.10 for the treatment group and 60.70 ± 1.50 for the placebo group ($P = 0.058$); after the intervention these values were 68.70 ± 1.90 and 51.60 ± 2.20 ($P \leq 0.001$), respectively.[32]

A similar finding was reported in a study by Lee et al[39] in which the QOL of patients with CRC was measured using the FACT measurement tool, version 4. The following scales were selected: General FACT scores (FACT-G), CRC-related FACT scores (FACT-C), fatigue-related FACT scores (FACT-F), and FACT-neurological symptoms (FACT-NTX) scores. In the probiotics group, which received a Lacidofil preparation containing *L. rhamnosus* and *L. acidophilus* bacterial culture (2×10^9 CFU), maltodextrin, magnesium stearate, and ascorbic acid, the QOL values associated with FACT-C scores were (± SD) 19.79 ± 4.66 vs 21.18 ± 3.67 ($P = 0.04$). FACT-F scores were [medians (range)] 43.00 (36.50–45.50) vs 44.50 (38.50–49.00; $P = 0.02$), and PHQ-9 scores were 3.00 (0–8.00) vs 1.00 (0–3.00; $P = 0.01$) for 0 weeks vs 12 weeks, respectively, which indicated a significant improvement after 12 weeks of treatment. The improvements in functional well-being scores ($P = 0.04$) and FACT-C scores ($P = 0.04$) between placebo and probiotic groups were significantly different after 12 weeks of treatment.[39] The evaluation of QOL related to gastrointestinal function at the first 6 months after surgery, using the validated Gastrointestinal Quality of Life Index questionnaire, showed that postoperatively, the synbiotics group's values were better than those of the control group (6 months:[± SEM] 79.23 ± 1.82 vs 72.75 ± 1.85, $P = 0.01$; 3 months: 77 ± 1.7 vs 72.5 ± 1.73, $P = 0.03$; and 1 month: 77 ± 1.67 vs 71.36 ± 1.69, $P = 0.01$).[45] The common factor among the studies is that they all used probiotic interventions that involved *Lactobacillus* and *Bifidobacterium* strains and ≥10 doses were administered. Extended consumption of probiotics/synbiotics promote better QOL and psychological well-being in patients with CRC and survivors, as well as reducing readmission, hospitalization time, and the healing process.

Death

Two RCTs reported deaths of some patients involved in the clinical trials. Generally, the mortality rates were low and occurred mainly in the control/placebo group due to escalated and severe postoperative complications such as pneumonia and bacteremia. The mortality rate 30 days after surgery in the placebo group was 2.9% and in the probiotic group it was 1.5% ($P = 1.000$) after a short duration of perioperative administration of a commercially available probiotic (VSL #3) containing *B. longum, L. acidophilus, Streptococcus thermophilus*, *L. plantarum, B. breve*, *B. infantis, L. bulgaricus,* and *L. paracasei* at 112.5 billion CFU/capsule.[29] Another study reported 1 death due to severe pneumonia escalating to respiratory failure in the placebo group ($P = 0.31$) and no death was recorded in the probiotic group, which was administered Familact; each capsule contains *L. casei, L. acidophilus*, *L. plantarum, B. breve* (1.75×10^9CFU), *B. longum*, and *S. thermophilus.*[34]

DISCUSSION

Probiotics are described as live microorganisms that provide such beneficial health effects as maintaining gut microbial balance, modulating the immune system, and improving general QOL of consumers.[10] Some other reviews have highlighted the beneficial effects of probiotics in preclinical studies and in clinical trials, and the authors came to similar conclusions that probiotics have therapeutic and preventive effect on CRC carcinogenesis.[20,71] However, several studies have shown that the administration of probiotics in patients undergoing colorectal surgery does not affect the occurrence of postoperative infections.[72] He et al[23] performed a meta-analysis of 6 RCTs with 361 patients, the results of which suggested the use of probiotics perioperatively did not minimize the occurrence of complications such as incisional infection, bacteremia, and anastomotic leaks. These findings most likely were due to the small sample size and remarkable heterogeneity that may have affected the reliability and validity of the conclusions.

The findings from 23 studies in the present review, which included 2457 participants, indicate that the use of diverse strains of probiotics improves the overall health status of patients with CRC and enables them to manage the symptoms as well as ameliorate the side effects of chemotherapeutics against CRC. Most primary studies assessed the administration of probiotic mainly from *Lactobacillus* and/or *Bifidobacterium* because they have clear evidence of being safe.[73] Furthermore, most of the studies assessed >1 outcome of the use of probiotics for patients with CRC, as can be seen in Table 2. There was no serious variation in age range (17–90 years) of the participants involved in the included studies. Although the included studies showed a wide range of methodical variability, some similar outcomes were reported.

The reports from RCTs that studied the influence of probiotics on immune modulation were reviewed and the data indicated probiotics may enhance the immune response by stimulating anti-inflammatory

factors and promoting the production of antioxidant enzymes. Results indicated the interaction of probiotics with toll-like receptors led to the inhibition of NF-κB in macrophages, activation of anti-inflammatory cytokines, increment in the level of TNF-α in epithelial cells, and the production of IL-8 required for recruitment of neutrophils. Some species of *Lactobacillus* promote the regulatory T cells and the activities of antibacterial phagocytic of peripheral blood neutrophils and natural killer cells.

Approximately 53% of the included studies used a mixture of probiotics as an intervention. The use of probiotic mixture (containing multiple strains of probiotic bacteria) and synbiotics is seen to be consistent with better outcomes such as reduction of SSI incidence and fewer noninfectious complications than administration of a single strain of a probiotic. This is because probiotics increase the diversity of the intestinal microbiota, promoting synergy between probiotic strains for better results and promoting a healthy normal microbiota by multistrain probiotics. On the contrary, it is worth noting that not all types of probiotic mixtures can produce a positive benefit. A published systematic review of 72 primary studies concluded that certain probiotic products such as *Saccharomyces boulardii* or *L. rhamnosus* GG, when used postoperatively in patients with CRC, may facilitate the risk of complications in patients with organ disorders. It is possible that certain probiotics could result in bacteremia or fungemia in patients.[74] Therefore, certain probiotics may work differently in various patients and clinical conditions. No obvious adverse reactions were reported in any of the primary studies included in this systematic review; however, the need for regular monitoring of side effects is critical during the consumption of probiotics in patients with CRC.

The findings in this systematic review also highlight the importance of preoperative administration of probiotics or synbiotics as well as postoperative administration of probiotics, especially for patients with CRC who have invasive surgeries and are on a chemotherapy regimen. Infection during abdominal surgery, which is considered is a risk factor for increased morbidity and mortality rates of patients with CRC, can be reduced by administering probiotics to patients before their surgery. Evidence suggested that administration of probiotics before and after surgery, as well as constantly during and after chemotherapy, increases the chance of the probiotics to survive in the gut to be able to aid in reduction of tumor size, restriction of BT, reduction of inflammatory biomarkers, and ameliorate the side effects of chemotherapy.

The administeration of probiotics in the included studies was via different forms, such as in a capsule or powder, or infused in oatmeal-based drinks, kefir, or other probiotic-containing drinks. Generally, commercial probiotic formulations come in diverse forms and can contain up to 10^6–10^9 CFU of viable organisms. The findings also suggested that the form of probiotic administration does not influence the outcome of probiotic/symbiotic ingestion. However, dosage and duration of probiotic administration play an important role in the outcome of probiotic supplementation. Insignificant effects of probiotics reported in patients with CRC were mostly due to the short duration of probiotic intervention or ineffective dosage administered. Most of the included studies suffered from the limitation of a smaller sample size as well as relative short duration of probiotic use, premature termination of study, and the effects of bowel-cleansing mechanisms or laxatives given to patients before undergoing surgery. All these factors reduced the ability of some of these studies to detect clinical significance.

Probiotics are generally considered safe to be consumed by people of all ages except for those with a compromised immune system. Probiotics can alleviate diarrhea symptoms in children and provide relief from inflammatory bowel disease. Probiotic use also provides relief from constipation in people of all ages.[75]

The use of probiotics and synbiotics provides numerous health benefits, as seen in this systematic review. However, there are several challenges, such as poor lifestyle choices, genetic variations, and severe reactions to prolonged chemotherapy and radiotherapy, which can affect the use of probiotics against CRC negatively.

Another limitation is that prolonged use of some probiotics could potentially have negative effects on patients with CRC who have comorbidities. A recent study showed that prolonged probiotic supplementation that contained *Lactobacillus* spp. could put patients at risk for endocarditis, especially in immunosuppressed individuals.[76] Long-term use of probiotics could also lead to a horizontal transfer of antibiotic resistance from the probiotics to other microorganisms in the gut, such as seen in the transfer of antibiotic-resistant genes from *L. lactis* to *Enterococcus faecalis*.[77] In addition, prolonged use of probiotics in patients who have a weak immune system could lead to opportunistic infection. Although the risk of *Bifidobacteria*- or *Lactobacillus*-associated opportunistic infection is very low, strains of *Streptococcus* and *Enterococcus* have been linked to some opportunistic infections.[78]

Currently, there are few ongoing clinical trials investigating the effect of probiotics in patients with CRC (Table 4). The outcomes of these clinical trials include QOL of patients, the level of immunoglobulins (IgA, IgG, and IgM), and the proportion of patients with

tumor size reduction and postoperative complications after the supplementation of probiotics in patients with CRC. The findings of these ongoing studies will add more information about the role of probiotics in patients with CRC.

In the future, the use of probiotics against CRC should be able to broaden and become more personalized in treatment of patients with CRC. Dietitians, nutritionists, and general practitioners should also recommend intake of probiotic foods or supplements more frequently to reduce the risk of CRC development, and the administration of probiotics should commence at the first diagnosis of colon polyps.

Because the underlying mechanisms by which probiotic supplementation ameliorates CRC are still not fully understood, more preclinical studies as well as an extensive metagenomic and metabolomic research need are needed. Randomized clinical trials could also be carried out comparing the action of different probiotic strains instead of comparing with placebo or a control group, because this will provide more information regarding the most effective probiotic strains. Metagenomic and metabolomic studies should also be done with patients with CRC, especially those who undergo surgical resections.

CONCLUSION

that the findings from this review indicate probiotics and synbiotics are beneficial for patients with CRC regardless of the stage of cancer. Probiotics administration in patients with CRC not only reduce risk of infection after surgery but also reduce tumor incidence in the long run, modulate the immune system as well as improve the general QOL, and alleviate the side effects of conventional treatment procedures used to combat CRC. There is an opportunity for probiotics to be used in the mainstream in combination with the current treatment as an alternative therapy in the fight against CRC. Novel probiotics should also be explored and used as interventions instead of limiting the use of probiotics to only species of *Lactobacillus* and/or *Bifidobacterium*. More RCTs with larger sample sizes and metabolomic studies are needed to further understand the physiological, cellular, and molecular effects of probiotics, as well as their interactions with chemotherapeutics agents.

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Availability of data and materials. All data analyzed during this study are included in the figures and tables in the manuscript.

Declaration of interest. The authors declare that they have no competing interests.

SUPPORTING INFORMATION

The following Supporting Information is available through the online version of this article at the publisher's website.

Table S1 **PRISMA checklist**

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 v1.0.*IARC CancerBase* 2013. Doi: 978-92-832-2447-1
2. dos Reis SA, da Conceição LL, Siqueira NP, et al. Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. *Nutr Res*. 2017;37:1–19.
3. Derry MM, Raina K, Agarwal C, et al. Identifying molecular targets of lifestyle modifications in colon cancer prevention. *Front Oncol*. 2013;3:119–139. MAY00119. doi:10.3389/fonc.2013.00119.
4. Ho JTK, Chan GCF, Li JCB. Systemic effects of gut microbiota and its relationship with disease and modulation. *BMC Immunol*. 2015;16:21.
5. Saus E, Iraola-Guzmán S, Willis JR, et al. Microbiome and colorectal cancer: roles in carcinogenesis and clinical potential. Review. *Mol Aspects Med*. 2019;69:93–106.
6. Ding C, Tang W, Fan X, et al. Intestinal microbiota: a novel perspective in colorectal cancer biotherapeutics. *Onco Targets Ther*. 2018;11:4797–4810.
7. Sivamaruthi BS, Kesika P, Chaiyasut C. The role of probiotics in colorectal cancer management. *Evidence-Based Complementary and Alternative Medicine*. 2020:2020
8. Mego M, Holec V, Drgona L, et al. Probiotic bacteria in cancer patients undergoing chemotherapy and radiation therapy. *Complement Ther Med*. 2013;21:712–723. doi:10.1016/j.ctim.2013.08.018
9. Hill C, Guarner F, Reid G, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11:506–514.
10. Shi LH, Balakrishnan K, Thiagarajah K, et al. Beneficial properties of probiotics. *Trop Life Sci Res*. 2016;27:73–90.
11. Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol*. 2017;14:491–502.
12. Xie XL, He YQ, Li H, et al. Effects of prebiotics on immunologic indicators and intestinal microbiota structure in perioperative colorectal cancer patients. *Nutrition*. 2019;61:132–142.
13. Fotiadis CI, Stoidis CN, Spyropoulos BG, et al. Role of probiotics, prebiotics and synbiotics in chemoprevention for colorectal cancer. *World J Gastroenterol*. 2008;14:6453–6457. doi:10.3748/wjg.14.6453
14. Liong MT. Roles of probiotics and prebiotics in colon cancer prevention: postulated mechanisms and in-vivo evidence. *Int J Mol Sci*. 2008;9:854–863. doi:10.3390/ijms9050854
15. Dronamraju SS, Coxhead JM, Kelly SB, et al. Cell kinetics and gene expression changes in colorectal cancer patients given resistant starch: a randomised controlled trial. *Gut*. 2009;58:413–420.
16. Cruz B, Sarandy MM, Messias AC, et al. Preclinical and clinical relevance of probiotics and synbiotics in colorectal carcinogenesis: a systematic review. *Nutr Rev*. 2020;78:667–687.
17. Hinnebusch BF, Meng S, Wu JT, et al. The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation. *J Nutr*. 2002;132:1012–1017.
18. Perrin P, Cassagnau E, Burg C, et al. An interleukin 2/sodium butyrate combination as immunotherapy for rat colon cancer peritoneal carcinomatosis. *Gastroenterology*. 1994;107:1697–1708.
19. Andoh A, Tsujikawa T, Fujiyama Y. Role of dietary fiber and short-chain fatty acids in the colon. *Curr Pharm Des*. 2003;9:347–358.

20. de Ameida Brasiel PG, Luquetti SCPD, MdCG P, et al. Preclinical evidence of probiotics in colorectal carcinogenesis: a systematic review. *Digest Dis Sci*. 2020;65:3197–3210.
21. Lu DX, Yan J, Liu F, et al. Probiotics in preventing and treating chemotherapy-induced diarrhea: a meta-analysis. *Asia Pac J Clin Nutr*. 2019;28:701–710
22. Wang L, Guo M-J, Gao Q, et al. The effects of probiotics on total cholesterol: a meta-analysis of randomized controlled trials. *Medicine*. 2018;97: 1–8.
23. He D, Wang HY, Feng JY, et al. Use of pro-/synbiotics as prophylaxis in patients undergoing colorectal resection for cancer: a meta-analysis of randomized controlled trials. *Clin Res Hepatol Gastroenterol*. 2013;37:406–415.
24. Moher D, Shamseer L, Clarke M, PRISMA-P Group, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev*. 2015;4:1.
25. Dikeocha IJ, Al-Kabsi AM, Hussin S, et al. Role of probiotics in patients with colorectal cancer: a systematic review protocol of randomised controlled trial studies. *BMJ Open*. 2020;10:E038128.
26. Huang X, Lin J, Demner-Fushman D. Evaluation of PICO as a knowledge representation for clinical questions. *Am Med Inform Assoc*. 2006;2006:359–363.
27. Consoli ML, da Silva RS, Nicoli JR, et al. Randomized clinical trial: impact of oral administration of *Saccharomyces boulardii* on gene expression of intestinal cytokines in patients undergoing colon resection. *JPEN J Parenter Enteral Nutr*. 2016;40:1114–1121. doi:10.1177/0148607115584387
28. Flesch AT, Tonial ST, Contu PC, et al. Perioperative synbiotics administration decreases postoperative infections in patients with colorectal cancer: a randomized, double-blind clinical trial. *Rev Col Bras Cir*. 2017;44:567–573.
29. Franko J, Raman S, Krishnan N, et al. Randomized trial of perioperative probiotics among patients undergoing major abdominal operation. *J Am Coll Surg*. 2019;229:533–540.e1.
30. Gao Z, Guo B, Gao R, et al. Probiotics modify human intestinal mucosa-associated microbiota in patients with colorectal cancer. *Mol Med Rep*. 2015;12:6119–6127.
31. Gianotti L, Braga M, Morelli L, et al. Role of probiotics in colorectal surgery: a prospective randomized double-blind pilot study. *Nutr Ther Metab*. 2008;26:190–198.
32. Golkhalkhali B, Rajandram R, Paliany AS, et al. Strain-specific probiotic (microbial cell preparation) and omega-3 fatty acid in modulating quality of life and inflammatory markers in colorectal cancer patients: a randomized controlled trial. *Asia Pac J Clin Oncol*. 2018;14:179–191.
33. Ishikawa H, Akedo I, Otani T, et al. Randomized trial of dietary fiber and *Lactobacillus casei* administration for prevention of colorectal tumors. *Int J Cancer*. 2005;116:762–767.
34. Kakaei F, Shahrasbi M, Kermani TA, et al. Assessment of probiotic effects on colorectal surgery complications: a double blinded, randomized clinical trial. *Biomed Res Ther*. 2019;6:3067–3072.
35. Komatsu S, Sakamoto E, Asahara T, et al. Effects of synbiotics on ileal microbiota. *Indian J Med Res*. 2018;147:58–65.
36. Komatsu S, Sakamoto E, Norimizu S, et al. Efficacy of perioperative synbiotics treatment for the prevention of surgical site infection after laparoscopic colorectal surgery: a randomized controlled trial. *Surg Today*. 2016;46:479–490.
37. Kotzampassi K, Stavrou G, Damoraki G, et al. A four-probiotics regimen reduces postoperative complications after colorectal surgery: a randomized, double-blind, placebo-controlled study. *World J Surg*. 2015;39:2776–2783.
38. Krebs B. Prebiotic and synbiotic treatment before colorectal surgery–randomised double blind trial. *Coll Antropol*. 2016;40:35–40.
39. Lee JY, Chu SH, Jeon JY, et al. Effects of 12 weeks of probiotic supplementation on quality of life in colorectal cancer survivors: a double-blind, randomized, placebo-controlled trial. *Digest Liver Dis*. 2014;46:1126–1132.
40. Liu ZH, Huang MJ, Zhang XW, et al. The effects of perioperative probiotic treatment on serum zonulin concentration and subsequent postoperative infectious complications after colorectal cancer surgery: a double-center and double-blind randomized clinical trial. *Am J Clin Nutr*. 2013;97:117–126.
41. Mangell P, Thorlacius H, Syk I, et al. *Lactobacillus plantarum* 299v does not reduce enteric bacteria or bacterial translocation in patients undergoing colon resection. *Dig Dis Sci*. 2012;57:1915–1924.
42. Mego M, Chovanec J, Vochyanova-Andrezalova I, et al. Prevention of irinotecan induced diarrhea by probiotics: a randomized double blind, placebo controlled pilot study. *Complement Ther Med*. 2015;23:356–362.
43. Mizuta M, Endo I, Yamamoto S, et al. Perioperative supplementation with Bifidobacteria improves postoperative nutritional recovery, inflammatory response, and fecal microbiota in patients undergoing colorectal surgery: a prospective, randomized clinical trial. *Biosci Microbiota Food Health*. 2016;35:77–87.
44. Roller M, Clune Y, Collins K, et al. Consumption of prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* has minor effects on selected immune parameters in polypectomised and colon cancer patients. *Br J Nutr*. 2007;97:676–684.
45. Theodoropoulos GE, Memos NA, Peitsidou K, et al. Synbiotics and gastrointestinal function-related quality of life after elective colorectal cancer resection. *Ann Gastroenterol*. 2016;29:56–62.
46. Topuz E, Derin D, Can G, et al. Effect of oral administration of kefir on serum proinflammatory cytokines on 5-FU induced oral mucositis in patients with colorectal cancer. *Invest New Drugs*. 2008;26:567–572.
47. Yang Y, Xia Y, Chen H, et al. The effect of perioperative probiotics treatment for colorectal cancer: short-term outcomes of a randomized controlled trial. *Oncotarget*. 2016;7:8432–8440.
48. Zaharuddin L, Mokhtar NM, Muhammad Nawawi KN, et al. A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer. *BMC Gastroenterol*. 2019;19:131.
49. Zhang JW, Du P, Gao J, et al. Preoperative probiotics decrease postoperative infectious complications of colorectal cancer. *Am J Med Sci*. 2012;343:199–205. doi:10.1097/MAJ.0b013e31823aace6
50. Sterne JA, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019;2019:366.
51. Schünemann HJ, Oxman AD, Higgins JP, et al. Presenting results and 'Summary of findings' tables. *Cochrane Handbk Syst Rev Interven*. 2008;5: 11.1–11.19.
52. Brozek J, Oxman A, Schünemann HG. 3.2 for Windows. Grading of Recommendations Assessment. *Development and Evaluation (GRADE) Working Group*. Hamilton, Canada: The GRADE Working Group; 2008.
53. Balshem H, Helfand M, Schünemann HJ, et al. GRADE guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol*. 2011;64:401–406.
54. Aisu N, Tanimura S, Yamashita Y, et al. Impact of perioperative probiotic treatment for surgical site infections in patients with colorectal cancer. *Exp Therapeut Med*. 2015;10:966–972.
55. Gianotti L, Morelli L, Galbiati F, et al. A randomized double-blind trial on perioperative administration of probiotics in colorectal cancer patients. *World J Gastroenterol*. 2010;16:167–175. doi:10.3748/wjg.v16.i2.167
56. Kee BK, Morris JS, Slack RS, et al. A phase II, randomized, double blind trial of calcium aluminosilicate clay versus placebo for the prevention of diarrhea in patients with metastatic colorectal cancer treated with irinotecan. *Support Care Cancer*. 2015;23:661–670.
57. Liang SF, Xu L, Zhang DS, et al. Effect of probiotics on small intestinal bacterial overgrowth in patients with gastric and colorectal cancer. *Turk J Gastroenterol*. 2016;27:227–232.
58. Liu Z, Li C, Huang M, et al. Positive regulatory effects of perioperative probiotic treatment on postoperative liver complications after colorectal liver metastases surgery: a double-center and double-blind randomized clinical trial. *BMC Gastroenterol*. 2015;15:34.
59. Sadahiro S, Suzuki T, Tanaka A, et al. Comparison between oral antibiotics and probiotics as bowel preparation for elective colon cancer surgery to prevent infection: prospective randomized trial. *Surgery*. 2014;155: 493–503.
60. Ohigashi S, Hoshino Y, Ohde S, et al. Functional outcome, quality of life, and efficacy of probiotics in postoperative patients with colorectal cancer. *Surg Today*. 2011;41:1200–1206. doi:10.1007/s00595-010-4450-6
61. Worthley DL, Le Leu RK, Whitehall VL, et al. A human, double-blind, placebo-controlled, crossover trial of prebiotic, probiotic, and synbiotic supplementation: effects on luminal, inflammatory, epigenetic, and epithelial biomarkers of colorectal cancer. *Am J Clin Nutr*. 2009;90:578–586.
62. Wu WT, Cheng HC, Chen HL. Ameliorative effects of konjac glucomannan on human faecal -glucuronidase activity, secondary bile acid levels and faecal water toxicity towards Caco-2 cells. *Br J Nutr*. 2011;105:593–600.
63. Gough IR, Clunie GJA, Bolton PM, et al. A trial of 5-fluorouracil and *Corynebacterium parvum* in advanced colorectal carcinoma. *Dis Colon Rectum*. 1979;22:223–227.
64. Souter RG, Gill PG, Morris PJ. A trial of nonspecific immunotherapy using systemic *C. parvum* in treated patients with Dukes B and C colorectal cancer. *Br J Cancer*. 1982;45:506–512.
65. Fung WY, Lye HS, Lim TJ, et al. Roles of probiotic on gut health. In: Liong MT, ed. *Probiotics*. Berlin, Heidelberg: Springer; 2011:139–165.
66. Song M, Chan AT. Environmental factors, gut microbiota, and colorectal cancer prevention. *Clin Gastroenterol Hepatol*. 2019;17:275–289.
67. Gill H, Prasad J. Probiotics, immunomodulation, and health benefits. In: Bösze Z, ed. *Bioactive Components of Milk*. New York, NY: Springer; 2008:423–454.
68. Liu PC, Yan YK, Ma YJ, et al. Probiotics reduce postoperative infections in patients undergoing colorectal surgery: a systematic review and meta-analysis. *Gastroenterol Res Pract*. 2017;2017:1–9.
69. Yang ZP, Wu Q, Liu YF, et al. Effect of perioperative probiotics and synbiotics on postoperative infections after gastrointestinal surgery: a systematic review with meta-analysis. *JPEN J Parenter Enteral Nutr*. 2017;41:1051–1062.
70. Zhang L, Yu J. Role of apoptosis in colon cancer biology, therapy, and prevention. *Curr Colorectal Cancer Rep*. 2013;9:331–340.
71. Eslami M, Yousefi B, Kokhaei P, et al. Importance of probiotics in the prevention and treatment of colorectal cancer. *J Cell Physiol*. 2019;234:17127–17143.
72. Jeppsson B, Mangell P, Thorlacius H. Use of probiotics as prophylaxis for postoperative infections. *Nutrients*. 2011;3:604–612.
73. Borriello S, Hammes W, Holzapfel W, et al. Safety of probiotics that contain lactobacilli or Bifidobacteria. *Clin Infect Dis*. 2003;36:775–780.

74. Whelan K, Myers CE. Safety of probiotics in patients receiving nutritional support: a systematic review of case reports, randomized controlled trials, and nonrandomized trials. *Am J Clin Nutr*. 2010;91:687–703.
75. Armstrong C. AAP reports on use of probiotics and prebiotics in children. *Am Fam Physician*. 2011;83:849.
76. Kothari D, Patel S, Kim S-K. Probiotic supplements might not be universally-effective and safe: a review. *Biomed Pharmacother*. 2019;111:537–547.
77. Aarts H, Margolles A. Antibiotic resistance genes in food and gut (non-pathogenic) bacteria. Bad genes in good bugs. *Front Microbiol*. 2015;5:754.
78. Huys G, Botteldoorn N, Delvigne F, et al. Microbial characterization of probiotics–Advisory report of the Working Group "8651 Probiotics" of the Belgian Superior Health Council (SHC). *Mol Nutr Food Res*. 2013;57:1479–1504.