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The role of Probiotics in colorectal cancer patients: A systematic review protocol of randomized controlled trial studies

Journal:	BMJ Open
Manuscript ID	bmjopen-2020-038128
Article Type:	Protocol
Date Submitted by the Author:	02-Mar-2020
Complete List of Authors:	Dikeocha, Ifeoma; Cyberjaya University College of Medical Sciences, Medical science Al-kabis, Abdelkodose; Cyberjaya University College of Medical Sciences, Medical science Hussin, Salasawati; Cyberjaya University College of Medical Sciences, Medical science Alshawsh, Mohammed; University of Malaya Faculty of Medicine, Pharmacology
Keywords:	Gastrointestinal tumours < ONCOLOGY, Microbiology < PATHOLOGY, Clinical trials < THERAPEUTICS





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The role of probiotics in colorectal cancer patients: A systematic review protocol of randomized controlled trial studies Dikeocha Ifeoma Julieth¹ Abdelkodose M Al-kabsi¹ Salasawati Hussin¹ Mohammed Abdullah Alshawsh^{2*} ¹ Faculty of Medicine, University of Cyberjaya, Persiaran Bestari 63000 Cyberjaya, Selangor Darul Ehsan, Malaysia. ² Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia * Correspondence: alshaweshmam@um.edu.my; alshaweshmam@yahoo.com *Telephone no. +60379674950 Dikeocha Ifeoma Julieth: 1812-0326@st.cyberjaya.edu.my ; ifeomadikeocha19@gmail.com Assistant Prof. Dr. Abdelkodose M Al-kabsi: abdelkodose@cyberjaya.edu.my Associate Prof. Dr. Salasawati Hussin: salasawati@cyberjaya.edu.my Associate Prof. Dr. Mohammed Abdullah Alshawsh: alshaweshmam@um.edu.my; alshaweshmam@yahoo.com Abstract Introduction: Colorectal cancer is one of the leading causes of cancer related morbidity worldwide and it has been reported to be associated with poor lifestyle habits which include excess tobacco and alcohol intake as well as genetics and age factors. Probiotics such as the Lactic acid bacteria and Bifidobacterium as well as probiotic containing foods (kombucha, kefir, miso etc.) have received lots of attention as anticancer agents for prevention and treatment. The effects of the administration of probiotics to colorectal cancer patients is the primary goal of this systematic review. The overall aim is to assess how the use of probiotics in colorectal cancer patients helps in the management of colorectal cancer and its effect on the diversity of gut microbiota. The final systematic review will provide a comprehensive evidence base for the use and efficacy of probiotics in colorectal cancer patient care. Methods and analysis: The systematic review, will be conducted by extensively searching different databases such as PubMed, Web of Science, Scopus, Wiley and ProQuest to identify randomized controlled trials (with no time frame) which relate to the administration of probiotics to colorectal cancer patients. The search strategy will include words like colorectal cancer, probiotics, Bifidobacterium, clinical trials etc. Two reviewers will independently review the studies and also search the reference lists of the eligible studies to obtain more references. Data will be extracted from the eligible studies using standardized data extraction form. After assessing the risk of bias, qualitative analysis will be used to synthesize the systematic review. Ethics and Dissemination: This is a protocol for a systematic review; therefore, it doesn't require any ethics approval. We intend to disseminate the protocol in a peer reviewed journal.

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3	34	Keywords: Probiotics; Colorectal Cancer; Randomized controlled trial studies; Clinical trial;
4	35	Colon neoplasms.
5	55	
6 7	36	Strengths and Limitations of this study:
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9	37	Strengths and Limitations of this study:
10	20	
11	38	• The findings from this systematic review will provide current insight on how
12	39	probiotics are used either alone or in combination to improve the quality of life of
13	55	colorectal cancer patients.
14	40	 This study will highlight the efficacy and the underlying mechanism of actions of probiotics against colorectal cancer
15		 The results from this review will promote the use of probiotics as an alternative
16	41	therapy for and management of colorectal cancer.
17		• There will be limitations inherent to any systematic review such as the lack of
18	42	information on outcome variables, as well as the assumption that the evaluation
19	43	techniques are consistent across studies.
20	45	 Only studies in English language will be included in this systematic review.
21 22	44	
22	•••	
23	45	Introduction:
25		
26	46	Colorectal cancer (CRC) refers to tumors that start in the colon and spreads all the way to the
27	47	rectum. Different types of colorectal polyps exist, but colorectal cancer usually develops from
28	48	adenomas. CRC is one of the very common causes of mortality amongst cancer patients
29	49	worldwide including developed and undeveloped countries but mostly in first world countries. It
30		is predicted that by 2035, over 25 million incidences of CRC will be discovered on a yearly
31	50	
32	51	basis. ¹ It is also estimated that over 376,000 new cases of colorectal cancer diagnosis as well as
33	52	approximately 200,000 deaths take place yearly in China. ²
34	50	Colorectel concernmented to be a silent biller eilment that may not be noticed in time until the
35	53	Colorectal cancer proves to be a silent killer ailment that may not be noticed in time until the
36 37	54	cancer has progressed significantly. Symptoms of CRC resemble symptoms of several ailments
38	55	and is easily misdiagnosed unless a colonoscopy is done. The symptoms of CRC include
39	56	unexplained anemia, unexplained weight loss, bloating, changes in the bowel movement habits,
40	57	bloody stool, vomiting and pelvic pain. It has been proven that the initiating events of CRC
41	58	include TP53 mutation in colorectal cancer associated with colitis (CAC) as well as mutation in
42	59	sporadic colorectal cancer (SCC). ³ Different causes of colorectal cancer have been examined
43		• · · · · · · · · · · · · · · · · · · ·
44	60	over the years from data collected in cohort-based studies and these findings resemble studies
45	61	carried out in animal models, The common conclusion is that age, lifestyle choices such as
46	62	smoking and excessive alcohol intake which can lead to obesity or diabetes, as well as genetic
47	63	risk factors, contribute to the development of CRC. ⁴⁵
48		
49 50	64	Colorectal cancer can also be inherited through the genes by inheriting mutated genes that trigger
50 51	65	tumor growth, but this only accounts for about 5% of colorectal cancer cases. ⁶ In addition,
52	66	different researchers in their studies have agreed that an increased number of opportunistic
53	67	bacteria which quickly turn pathogenic such as <i>Helicobacter pylori, Bacteroides fragilis,</i>
54	68	Helicobacter hepaticus, enterotoxigenic Escherichia coli, Fusobacterium nucleatum, and
55	00	neneoouerer nepuneus, emeroionizente Eschertentu con, r'usobuctertum nucleutum, allu
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Streptococcus bovis, can lead to the initiation of adenomas formation that lead to colorectal
 70 cancer.⁷

71 Colorectal cancer patients usually undergo surgery to remove cancerous polyps or to remove

 $\frac{7}{8}$ $\frac{71}{72}$ some part of their colon which have been affected (colon resection). Others undergo

9 73 chemotherapy or radiotherapy to treat CRC. These treatment options are sometimes unsuccessful

74 or lead to a myriad of severe side effects which increase hospital stay time and sometimes
 75 morbidity.⁸

Probiotics is redefined by the international scientific association for probiotics and prebiotics (ISAPP) as "live microorganisms which when administered in adequate amounts, confer a health benefit on the host".⁹ Probiotic microorganisms are special because they are capable of surviving in the human gastrointestinal tract before they get to the colon, where the majority of their metabolic activity is carried out. They include lactic acid producing bacteria (LAB) of the genera Lactobacillus and Bifidobacterium as well as Propionibacterium, Saccharomyces and are the major ingredients in yoghurts and other functional foods such as unfermented milks, cheese,

22 83 kefir, fermented milk.¹⁰

On the other hand, prebiotics are usually termed as non-digestible carbohydrates such as inulin and oligosaccharides, soy and resistant starch. Prebiotics is defined by ISAPP as "a substrate that is selectively utilized by host microorganisms to confer health benefit to the host".⁹ Prebiotics stimulate an increased growth of probiotics by providing a more favorable environment for their growth.¹¹ Leading to a gut environment that promotes the competitive dismissal of opportunistic and potentially pathogenic bacteria which could initiate the beginning of CRC.¹¹ Several studies have shown that the administration of both probiotics and prebiotics as a combination can aid increasingly in improving the conditions of CRC patients especially after colorectal surgery has been performed.^{12 13}

Probiotics have been utilized by the traditional healers for the prevention and treatment of different types of illneses from the simple stomach ache to intestinal neoplasia. In addition various experimental studies have shown that continious ingestion of probiotic bacteria can enhance the qualitative as well as quantitative components of the gut microbiota.¹⁴ In one instance, the ingestion of Lactobacillus acidophilus LA-11, Lactobacillus plantarum CGMCC 1258, and *Bifidobacterium longum* BL-88 (2.6×10^{14} [CFU]/d) for 16days, resulted in an increase in the diversification of gut microflora and microbial richness in patients suffering from CRC who have been scheduled for colorectomy. Eventually, the microbial flora makeup of these individuals improved to resemble that of individuals without CRC.¹⁵ Probiotic bacteria are able to diminish the total quantity of non beneficial disease causing bacteria found in the colon by numerous mechanisms, particulary as regards; rivarly for nutrients, growth factors, and adhesion of the probiotics onto the intestinal cells of the host.¹⁶ Some probiotic bacteria can produce antibacterial substances such as bacteriocins, hydrogen peroxide, lactic acid and reuterin, which decrease the growth or totally eradicate pathogenic bacteria from the colon. The very popular advantages of the consumption and use of probiotics in the management and treatment of diarrhea associated to anti-cancer chemotherapy revolves around the restoration to normal of the intestinal microbiota.¹⁷ The favourable altreation by probiotic bacteria in the makeup of the gut

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- microbiome is closely associated with the reduced risk of suffering from CRC in the future.¹⁸
 Production of short chain fatty acids (SCFA) by probiotics which leads to cell apoptosis is one of the aways through which probiotics reduce the proliferation of colorectal carcinaoma.¹⁹
 Based on our search on systematic reviews related to our topic, we found out that most of the
- 9 114 systematic reviews which have been done were not entirely specific to colorectal cancer,¹² and
 10 115 those that are specific to probiotics and colorectal cancer patients focus on one outcome either on
- 10 115 those that are specific to probiotics and colorectal cancer patients focus on one outcome either o postoperative complications,²⁰ surgical site infection,²¹ diahreaa from chemotherapy.²² We see
- this as a limitiation of these studies, hence we intend to study more than one outcome in order to
- 117 this as a limitation of these studies, hence we intend to study more than one outcome in order
 14 118 get a wholisitc idea of how probiotics administration affect colorectal cancer patients who are
- recieving different types of treatment on different levels.

17 120 **Review Aim**

121 To systematically review, assess, and summarize and interpret clinical trials studies on how the
 122 use of probiotics compared to placebo in colorectal cancer patients in helps in the treatment, and
 123 management of colorectal cancer. In addition, this study will critically summarize how probiotics

administration in CRC patients affect the diversity of gut microbiome and patient quality of life.

24 25 125 Methods and Analysis

This systematic review protocol goes in accordance to the Preferred Reporting Items for
 Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines.²³

²⁹ 30 128 Eligibility Criteria for Included Studies

The inclusion criteria include studies on colorectal cancer patients who are were treated with chemotherapy, radiotherapy or surgery. The included studies must be carried out as randomized controlled trials with either a comparator group, control group or placebo group. Details of the inclusion and exclusion criteria that will be used in the systematic review is in Table 1.

Table 1. Eligibility criteria based on PICOS model
 Table 1. Eligibility criteria based on PICOS model

Items based on PICOS model	Eligibility criteria
i. Population, or participants and conditions of interest	Humans, any age, diagnosed with colorectal cancer or colon or rectal cancer and have been treated with probiotics as an intervention and this will include:
	Colorectal cancer patients who have had colorectal surgery or colon resection or haven't had surgery. Colorectal cancer patients who had or are still undergoing chemotherapy or radiotherapy or not.
ii. Interventions or exposures	Probiotics of any kind (e.g. <i>Lactobacillus,</i> <i>Bifidobacterium, Propionibacterium, Saccharomyce</i> etc.) used on its own or in combination with other probiotics or combination with prebiotics such as inulin or resistant starch etc.
iii. Comparisons or control groups	Placebos, or healthy people of any age, without

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 9 20 21 22 23 24 25 26 27 28 29	
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48 49	144
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52 53	146 147
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	Baseline comparison of patients before the intervention
iv. Outcomes of interest	 Primary outcomes: Effects of probiotics on the diversity of human gut microbiota. Effects of probiotics on inflammatory biomarkers relevant to CRC. Immunoregulatory action of probiotics. Secondary outcomes: Patient status (improvement/ no improvement of colorectal carcinoma) after administration of probiotics. Prognosis such as imaging to compare the size of cancer tumour before and after intervention General health and improvement in quality of life of the patient Adverse events such as morbidity and mortality
v. Study designs	Clinical trials, randomized clinical trials.
vi. Other exclusion criteria	 Articles not in English language. Reviews Animal or <i>in vitro</i> work done with probiotics. Studies not about colorectal cancer or rectal or colon cancer. Studies not testing the role of probiotics on
	colorectal cancer or rectal or colon cancer patients.
Search strategy	colorectal cancer or rectal or colon cancer
The relevant studies will be identified us Randomized controlled trials will be iden and ProQuest. The search results will be bibliography of all included randomized	colorectal cancer or rectal or colon cancer patients. ing standard search terms for individual databases. ntified from PubMed, Web of Science, Scopus, Wiley filtered to identify studies only in English. The controlled trials will be reviewed to identify any trials . This will be done independently by two reviewers.
The relevant studies will be identified us Randomized controlled trials will be iden and ProQuest. The search results will be bibliography of all included randomized missed during the initial database search	colorectal cancer or rectal or colon cancer patients. ing standard search terms for individual databases. ntified from PubMed, Web of Science, Scopus, Wiley filtered to identify studies only in English. The controlled trials will be reviewed to identify any trials . This will be done independently by two reviewers. by Boolean AND/OR operators:
The relevant studies will be identified us Randomized controlled trials will be iden and ProQuest. The search results will be bibliography of all included randomized missed during the initial database search Search terms will be used and connected The search syntax for PubMed will inclu	colorectal cancer or rectal or colon cancer patients. ing standard search terms for individual databases. ntified from PubMed, Web of Science, Scopus, Wiley filtered to identify studies only in English. The controlled trials will be reviewed to identify any trials . This will be done independently by two reviewers. by Boolean AND/OR operators:
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The relevant studies will be identified us Randomized controlled trials will be iden and ProQuest. The search results will be bibliography of all included randomized missed during the initial database search Search terms will be used and connected The search syntax for PubMed will inclu 1. Probiotic* OR Lactobacillus OR Bifid OR "Bacillus coagulans"	colorectal cancer or rectal or colon cancer patients. ing standard search terms for individual databases. ntified from PubMed, Web of Science, Scopus, Wiley filtered to identify studies only in English. The controlled trials will be reviewed to identify any trials . This will be done independently by two reviewers. by Boolean AND/OR operators: ide: lobacterium OR Propionibacterium OR Saccharomyce ectal
The relevant studies will be identified us Randomized controlled trials will be iden and ProQuest. The search results will be bibliography of all included randomized missed during the initial database search Search terms will be used and connected The search syntax for PubMed will inclu 1. Probiotic* OR Lactobacillus OR Bifid OR "Bacillus coagulans" 2. colon OR colorectal OR colonic OR re 3. cancer OR neopla* OR tumo* OR car	colorectal cancer or rectal or colon cancer patients. ing standard search terms for individual databases. ntified from PubMed, Web of Science, Scopus, Wiley filtered to identify studies only in English. The controlled trials will be reviewed to identify any trials . This will be done independently by two reviewers. by Boolean AND/OR operators: nde: lobacterium OR Propionibacterium OR Saccharomyce ectal

149 6. #1 AND #4 AND #5

The search syntax for other databases will be using similar approach as PubMed or using thefollowing merged search terms:

152 (Probiotic OR Lactobacillus OR Bifidobacterium OR Propionibacterium OR Saccharomyces OR

- 153 "Bacillus coagulans") AND (colon OR colorectal OR colonic OR rectal) AND (cancer OR
- 1 154 neopla* OR tumo* OR carcinoma OR malignan*) AND ("clinical trial" OR "intervention study"
- ² 155 OR "RCT" OR "randomised controlled trial" OR "randomized controlled trial")

14 156 Selection process of included studies

The primary article screening will be carried out independently by two reviewers. Titles and abstract of the studies will be screened independently, and the selected studies will be divided into three groups: relevant, irrelevant and unsure. The studies which are categorized as irrelevant by both reviewers will then be eliminated from the review. The full text of the remaining studies will be then reviewed by both reviewers using the eligibility criteria and studies that meet all the criteria will be included. In case of discrepancy, the two reviewers will first meet to discuss their choices and a final decision will be made. If there is any misunderstanding or conflict a third opinion will be sought from the other reviewers and when an agreement is reached, a final

26 165 decision will be made.27

166 Data extraction and Analysis

Studies meeting the inclusion criteria will be processed for data extraction. Two authors (DIJ and MAA) will independently screen title and abstract, and then full text. The data will be extracted and recorded in a consistent way using standardized data extraction form. The following data will be extracted: study year, author/s, study title, number of participants, stage of colorectal cancer, type of probiotic used, dosage of intervention, duration of intervention, control or placebo used, primary outcomes, secondary outcomes, conclusion and limitation.

The search and study framework will be represented using PRISMA flow chart²⁴ and the numbers of all included and excluded studies will be reported and the reasons for exclusion of

numbers of all included and excluded studies will be reported and the reasons for exclusion of studies will be given.

42 176 Assessment of risk of bias of included studies 43

The risk of bias will be assessed according to the guidelines of the Cochrane Collaboration using ROB tool.²⁵ this tool will be based on the following domains: random sequence generation, allocation concealment, adequacy of blinding for participants, blinding of outcome assessment, incomplete outcome data and selective reporting, and other sources of bias. RoB 2.0 will be used for risk of bias assessment of included study via RevMan version 5.3 software.²⁶ Two reviewers independently will carry out the assessment and if there is any conflict, third opinion will be obtained from third partner.

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185 Strategy for data synthesis

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3	186	Initial screening of the relevant studies showed that the outcomes of the included studies are not
4 5	187	homogenous and cannot be pooled together, therefore meta-analysis most likely will not be
5 6	188	carried out. Instead, qualitative analysis will be used to synthesize the studies included in the
7	189	systematic review. The quality of all included studies will be assessed by using PRISMA
8 9	190	checklist ²⁴ to ensure that the included studies are of good quality and to ensure that there is no
9 10	191	publication bias.
11 12	192	Patients and public involvement
13 14	193	There will be no need to involve patients or members of the general public in the design of this
14	194	systematic review, and no patients or member of the public will be contacted in order to
16	195	complete the systematic review.
17 18	196	Ethics and dissemination
19 20	197	Findings of this systematic review will be published in a peer-reviewed publication and will be
21	198	presented at a professional conference. Because this is only a protocol, no ethical assessment is
22	199	required.
23 24 25	200	Author Contributions
26	201	DIJ and MAA contributed to the conception of the study. The systematic review protocol was
27	202	drafted by DIJ and was revised by MAA and AMA. The search strategy was developed by DIJ
28 29	203	and MAA and will be performed by DIJ and MAA, who will also independently screen the
30	204	potential studies, extract data from the included studies, assess the risk of bias and complete the
31	205	data synthesis. AMA and SH will arbitrate in cases of disagreement and ensure the absence of
32 33	206	errors. All authors reviewed approved the publication of the protocol.
34 35	207	Funding statement
36	208	This research received no specific grant from any funding agency in the public, commercial or
37	209	not-for-profit sectors.
38 39 40	210	Competing interests' statement: NONE
41 42	211	Competing interests' statement: NONE Word count: ≤ 2000 words
43 44	212	Data statement section: Not Applicable
45 46	213	
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24	284	
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Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the PRISMA-Preporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

31	, , , , ,			
32			Reporting Item	Page Number
33 34 35	Title		4	
36 37 38	Identification	<u>#1a</u>	Identify the report as a protocol of a systematic review	Page 1
39 40 41 42	Update	<u>#1b</u>	If the protocol is for an update of a previous systematic review, identify as such	N/A
43	Registration			
44 45 46 47 48		<u>#2</u>	If registered, provide the name of the registry (such as PROSPERO) and registration number	Under review by PROSPERO
49 50	Authors			
51 52 53 54 55	Contact	<u>#3a</u>	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Page 1
56 57 58 59 60	Contribution	<u>#3b</u> For pee	Describe contributions of protocol authors and identify the guarantor of the review er review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	Page 7

1 2	Amendments			
2 3 4 5 6 7 8 9		<u>#4</u>	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
10 11	Support			
12 13 14 15	Sources	<u>#5a</u>	Indicate sources of financial or other support for the review	Page 7
16 17	Sponsor	<u>#5b</u>	Provide name for the review funder and / or sponsor	N/A
18 19 20 21	Role of sponsor or funder	<u>#5c</u>	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	N/A
22 23	Introduction			
24 25 26 27	Rationale	<u>#6</u>	Describe the rationale for the review in the context of what is already known	Page 3-4
28 29 30 31 32	Objectives	<u>#7</u>	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 4
33 34 35	Methods			
35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54	Eligibility criteria	<u>#8</u>	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Page 4-5
	Information sources	<u>#9</u>	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	Page 5
	Search strategy	<u>#10</u>	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Page 5-6
55 56 57 58	Study records - data management	<u>#11a</u>	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 6
59 60		For pee	er review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

1 2 3 4 5 6	Study records - selection process	<u>#11b</u>	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Page 6
7 8 9 10 11 12 13	Study records - data collection process	<u>#11c</u>	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Page 6
14 15 16 17 18	Data items	<u>#12</u>	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Page 6
19 20 21 22 23 24	Outcomes and prioritization	<u>#13</u>	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Page 5
25 26 27 28 29 30 31	Risk of bias in individual studies	<u>#14</u>	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	Page 6
32 33 34 35	Data synthesis	<u>#15a</u>	Describe criteria under which study data will be quantitatively synthesised	Page 6
33 36 37 38 39 40 41 42 43	Data synthesis	<u>#15b</u>	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's T)	N/A
44 45 46 47	Data synthesis	<u>#15c</u>	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	N/A
48 49 50	Data synthesis	<u>#15d</u>	If quantitative synthesis is not appropriate, describe the type of summary planned	Page 7
51 52 53 54 55 56	Meta-bias(es)	<u>#16</u>	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	N/A
57 58 59 60	Confidence in cumulative	<u>#17</u> For pee	Describe how the strength of the body of evidence will be assessed (such as GRADE) er review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	N/A

evidence

None The PRISMA-P checklist is distributed under the terms of the Creative Commons Attribution License CC-BY 4.0. This checklist can be completed online using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

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The role of Probiotics in colorectal cancer patients: A systematic review protocol of randomized controlled trial studies

Journal:	BMJ Open
Manuscript ID	bmjopen-2020-038128.R1
Article Type:	Protocol
Date Submitted by the Author:	14-May-2020
Complete List of Authors:	Dikeocha, Ifeoma; Cyberjaya University College of Medical Sciences, Medical science Al-kabsi, Abdelkodose; Cyberjaya University College of Medical Sciences, Medical science Hussin, Salasawati; Cyberjaya University College of Medical Sciences, Medical science Alshawsh, Mohammed; University of Malaya Faculty of Medicine, Pharmacology
Primary Subject Heading :	Oncology
Secondary Subject Heading:	Oncology, Pharmacology and therapeutics, Public health
Keywords:	Gastrointestinal tumours < ONCOLOGY, Microbiology < PATHOLOGY, Clinical trials < THERAPEUTICS





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The role of probiotics in colorectal cancer patients: A systematic review protocol of randomized controlled trial studies Dikeocha Ifeoma Julieth¹ Abdelkodose M Al-kabsi¹ Salasawati Hussin¹ Mohammed Abdullah Alshawsh^{2*} ¹ Faculty of Medicine, University of Cyberjaya, Persiaran Bestari 63000 Cyberjaya, Selangor Darul Ehsan, Malaysia. ² Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia * Correspondence: alshaweshmam@um.edu.my; alshaweshmam@yahoo.com *Telephone no. +60379674950 Dikeocha Ifeoma Julieth: 1812-0326@st.cyberjaya.edu.my ; ifeomadikeocha19@gmail.com Assistant Prof. Dr. Abdelkodose M Al-kabsi: abdelkodose@cyberjaya.edu.my; abdelkodose@gmail.com Associate Prof. Dr. Salasawati Hussin: salasawati@cyberjaya.edu.my Associate Prof. Dr. Mohammed Abdullah Alshawsh: alshaweshmam@um.edu.my; alshaweshmam@yahoo.com Abstract Introduction: Colorectal cancer is one of the leading causes of cancer related morbidity worldwide and it has been reported to be associated with poor lifestyle habits which include excess tobacco and alcohol intake as well as genetics and age factors. Probiotics such as the Lactic acid bacteria and Bifidobacterium as well as probiotic containing foods (kombucha, kefir, miso etc.) have received lots of attention as anticancer agents for prevention and treatment. The effects of the administration of probiotics to colorectal cancer patients is the primary goal of this systematic review. The overall aim is to assess how the use of probiotics in colorectal cancer patients helps in the management of colorectal cancer and its effect on the diversity of gut microbiota. The final systematic review will provide a comprehensive evidence base for the use and efficacy of probiotics in colorectal cancer patient care. Methods and analysis: The systematic review, will be conducted by extensively searching different databases such as PubMed, Web of Science, Scopus, Wiley and ProQuest to identify randomized controlled trials (with no time frame) which relate to the administration of probiotics to colorectal cancer patients. The search strategy will include words like colorectal cancer, probiotics, Bifidobacterium, clinical trials etc. A systematic search of databases was performed between 17 and 20 January 2020. Two reviewers will independently review the studies and also search the reference lists of the eligible studies to obtain more references. Data will be extracted from the eligible studies using standardized data extraction form. After assessing the risk of bias, qualitative analysis will be used to synthesize the systematic review. **Ethics and Dissemination**: This is a protocol for a systematic review; therefore, it doesn't require any ethics approval. We intend to disseminate the protocol in a peer reviewed journal.

1 2		
2 3	35	Keywords: Probiotics; Colorectal Cancer; Randomized controlled trial studies; Clinical trial;
4 5	36	Colorectal neoplasms.
6 7	37	Strengths and Limitations of this study:
8 9	38	Strengths and Limitations of this study:
10 11	39	• The findings from this systematic review will provide current insight on how probiotics are used either alone or in combination to improve the quality of life of
12 13	40	 This study will highlight the efficacy and the underlying mechanism of actions of
14 15	41	probiotics against colorectal cancer
16	42	 The findings from this review will improve our knowledge of the beneficial effects of probiotics in prevention and management of colorectal cancer.
17 18	43	• There will be limitations inherent to any systematic review such as the lack of
19	15	information on outcome variables, as well as the assumption that the evaluation techniques are consistent across studies.
20	44	 Only studies in English language will be included in this systematic review.
21	45	
22 23	45	
24	46	Introduction:
25	47	Colorectal cancer (CRC) refers to tumors that start in the colon and spreads all the way to the
26		
27 28	48 40	rectum. Different types of colorectal polyps exist, but colorectal cancer usually develops from
28 29	49 50	adenomas. CRC is one of the very common causes of mortality amongst cancer patients
30	50	worldwide including developed and undeveloped countries but mostly in first world countries. It
31	51	is predicted that by 2035, over 25 million incidences of CRC will be discovered on a yearly
32	52	basis. ¹ It is also estimated that over 376,000 new cases of colorectal cancer diagnosis as well as
33 34	53	approximately 200,000 deaths take place yearly in China. ²
35	54	Colorectal cancer proves to be a silent killer ailment that may not be noticed in time until the
36	55	cancer has progressed significantly. Symptoms of CRC resemble symptoms of several ailments
37	56	and is easily misdiagnosed unless a colonoscopy is done. The symptoms of CRC include
38	57	unexplained anemia, unexplained weight loss, bloating, changes in the bowel movement habits,
39 40	58	bloody stool, vomiting and pelvic pain. It has been proven that the initiating events of CRC
41	59	include TP53 mutation in colorectal cancer associated with colitis (CAC) as well as mutation in
42	60	sporadic colorectal cancer (SCC). ³ Different causes of colorectal cancer have been examined
43	61	over the years from data collected in cohort-based studies and these findings resemble studies
44	62	carried out in animal models, The common conclusion is that age, lifestyle choices such as
45 46		
40 47	63	smoking and excessive alcohol intake which can lead to obesity or diabetes, as well as genetic risk fortune contribute to the development of $CPC 45$
48	64	risk factors, contribute to the development of CRC. ^{4 5}
49 50	65	Colorectal cancer can also be inherited through the genes by inheriting mutated genes that trigger
50 51	66	tumor growth, but this only accounts for about 5% of colorectal cancer cases. ⁶ In addition,
52	67	different researchers in their studies have agreed that an increased number of opportunistic
53	68	bacteria which quickly turn pathogenic such as Helicobacter pylori, Bacteroides fragilis,
54	69	Helicobacter hepaticus, enterotoxigenic Escherichia coli, Fusobacterium nucleatum, and
55		
56		
57 58		2

Streptococcus bovis, can lead to the initiation of adenomas formation that lead to colorectal
 cancer.⁷

72 Colorectal cancer patients usually undergo surgery to remove cancerous polyps or to remove

 $\frac{7}{8}$ $\frac{72}{73}$ some part of their colon which have been affected (colon resection). Others undergo

9 74 chemotherapy or radiotherapy to treat CRC. These treatment options are sometimes unsuccessful

or lead to a myriad of severe side effects which increase hospital stay time and sometimes
 morbidity.⁸

Probiotics is redefined by the international scientific association for probiotics and prebiotics (ISAPP) as "live microorganisms which when administered in adequate amounts, confer a health benefit on the host".⁹ Probiotic microorganisms are special because they are capable of surviving in the human gastrointestinal tract before they get to the colon, where the majority of their metabolic activity is carried out. They include lactic acid producing bacteria (LAB) of the genera Lactobacillus and Bifidobacterium as well as Propionibacterium, Saccharomyces and are the major ingredients in yoghurts and other functional foods such as unfermented milks, cheese,

22 84 kefir. fermented milk.¹⁰

On the other hand, prebiotics are usually termed as non-digestible carbohydrates such as inulin and oligosaccharides, soy and resistant starch. Prebiotics is defined by ISAPP as "a substrate that is selectively utilized by host microorganisms to confer health benefit to the host".⁹ Prebiotics stimulate an increased growth of probiotics by providing a more favorable environment for their growth.¹¹ Leading to a gut environment that promotes the competitive dismissal of opportunistic and potentially pathogenic bacteria which could initiate the beginning of CRC.¹¹ Several studies have shown that the administration of both probiotics and prebiotics as a combination can aid increasingly in improving the conditions of CRC patients especially after colorectal surgery has been performed.^{12 13}

Probiotics have been utilized by the traditional healers for the prevention and treatment of different types of illneses from the simple stomach ache to intestinal neoplasia. In addition various experimental studies have shown that continious ingestion of probiotic bacteria can enhance the qualitative as well as quantitative components of the gut microbiota.¹⁴ In one instance, the ingestion of Lactobacillus acidophilus LA-11, Lactobacillus plantarum CGMCC 1258, and *Bifidobacterium longum* BL-88 (2.6×10^{14} [CFU]/d) for 16days, resulted in an increase in the diversification of gut microflora and microbial richness in patients suffering from CRC who have been scheduled for colorectomy. Eventually, the microbial flora makeup of these individuals improved to resemble that of individuals without CRC.¹⁵ Probiotic bacteria when consumed in adequate quantities are able to diminish the total quantity of non beneficial disease causing bacteria found in the colon by numerous mechanisms, particulary as regards; rivalry for nutrients, growth factors, and adhesion of the probiotics onto the intestinal cells of the host.¹⁶ Ingestion of probiotic also inhibits the activity of pathobionts such as *Clostridium perfringens* and Klebsiella pneumonia which are potential pathogenic microorganisms and could also be symbiotic microorganisms under certain gut environment conditions.¹⁷ Some probiotic bacteria can produce antibacterial substances such as bacteriocins, hydrogen peroxide, lactic acid and reuterin, which decrease the growth or totally eradicate pathogenic bacteria from the colon. The

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111 very popular advantages of the consumption and use of probiotics in the management and 112 treatment of diarrhea associated to anti-cancer chemotherapy revolves around the restoration to 113 normal of the intestinal microbiota.¹⁸ The favourable altreation by probiotic bacteria in the 114 makeup of the gut microbiome is closely associated with the reduced risk of suffering from CRC 115 in the future.¹⁹ Production of short chain fatty acids (SCFA) by probiotics which leads to cell 116 apoptosis is one of the aways through which probiotics reduce the proliferation of colorectal

- apoptosis is one of the aways through which problems reduce the promeration of confectal
 11 117 carcinaoma.²⁰ Scientific eveidence by various *in-vitro and in-vivo* studies have concluded that
- 12 118 various strains of probiotics possess anti-carcinogenic properties via different mechanisms.²¹²²

Based on our search on systematic reviews related to our topic, we found out that most of the systematic reviews which have been done were not entirely specific to colorectal cancer.¹² and those that are specific to probiotics and colorectal cancer patients focus on one outcome either on postoperative complications,²³ surgical site infection,²⁴ diahrrea from chemotherapy.²⁵ We see this as a limitation of these studies, hence we intend to study more than one outcome in order to get a holisitc idea of how probiotics administration affect colorectal cancer patients who are receiving different types of treatment on different levels. As we will asses several outcomes, these outcomes will be categorized and discussed based on if they are primary or secondary outcomes. Previously published reviews related to probiotics mostly investigated its effect on CRC and the mechanisms through which probiotics ameliorate CRC using diverse models including pre-clinical studies, and in-vitro studies.^{26 27} Some reviews also focused more on the use of specific probiotic as anticancer adjuvant.²⁸ Our systematic review is unique and different from other reviews in which we intend to include only randomized clinical trial studies (RCT) and asses the effects of the administration of various types of probiotics on colorectal cancer

³¹ 133 patients.

³³ 134 **Review Aim** 34

To systematically review, assess, and summarize and interpret clinical trials studies on how the
 trials studies on how the
 use of probiotics compared to placebo in colorectal cancer patients in helps in the treatment, and
 management of colorectal cancer. In addition, this study will critically summarize how probiotics
 administration in CRC patients affect the diversity of gut microbiome and patient quality of life.

41 139 Methods and Analysis

This systematic review protocol goes in accordance to the Preferred Reporting Items for
 Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines.²⁹

46 142 Eligibility Criteria for Included Studies

The inclusion criteria include studies on colorectal cancer patients who are were treated with
the inclusion criteria include studies on colorectal cancer patients who are were treated with
chemotherapy, radiotherapy or surgery. The included studies must be carried out as randomized
controlled trials with either a comparator group, control group or placebo group. Details of the
inclusion and exclusion criteria that will be used in the systematic review is in Table 1.

53 147

Items based on PICOS model	Eligibility criteria
i. Population, or participants and conditions of interest	Humans, any age, diagnosed with colorectal cancer or with colon cancer or rectal cancer and have been treated with probiotics as an intervention and this w include:
	Colorectal cancer patients who have had colorectal surgery or colon resection or haven't had surgery. Colorectal cancer patients who had or are still undergoing chemotherapy or radiotherapy or not.
" T	
ii. Interventions or exposures	Probiotics of any kind (e.g. <i>Lactobacillus,</i> <i>Bifidobacterium, Propionibacterium, Saccharomyce</i> etc.) used on its own or in combination with other probiotics or combination with prebiotics such as inulin or resistant starch etc.
iii. Comparisons or control groups	Placebos, or healthy people of any age, without colorectal cancer. Baseline comparison of patients before the intervention
iv. Outcomes of interest	 Primary outcomes: Effects of probiotics on the diversity of human gut microbiota. Effects of probiotics on inflammatory biomarkers relevant to CRC. Immunoregulatory action of probiotics. Secondary outcomes: Patient status (improvement/ no improvement of colorectal carcinoma) after administration of probiotics. Prognosis such as imaging to compare the size of cancer tumour before and after intervention General health and improvement in quality of life the patient Adverse events such as morbidity and mortality
v. Study designs	Clinical trials, randomized clinical trials.
vi. Other exclusion criteria	 Articles not in English language. Reviews Animal or <i>in vitro</i> work done with probiotics. Studies not about colorectal cancer or recta or colon cancer. Studies not testing the role of probiotics or colorectal cancer or rectal or colon cancer patients.

149 Table 1. Eligibility criteria based on PICOS model

1		
2 3		
4	152	Search strategy
5	153	The relevant studies will be identified using standard search terms for individual databases.
6 7	154	Randomized controlled trials will be identified from PubMed, Web of Science, Scopus, Wiley
7 8	155	and ProQuest. The search results will be filtered to identify studies only in English. The
9	156	bibliography of all included randomized controlled trials will be reviewed to identify any trials
10	157	missed during the initial database search. This will be done independently by two reviewers.
11 12	158	Search terms will be used and connected by Boolean AND/OR operators:
12 13 14	159	The search syntax for PubMed will include:
15	160	1. Probiotic* OR Lactobacillus OR Bifidobacterium OR Propionibacterium OR Saccharomyces
16 17	161	OR "Bacillus coagulans"
18 19	162	2. colon OR colorectal OR colonic OR rectal
20 21	163	3. cancer OR neopla* OR tumo* OR carcinoma OR malignan*
22	164	4. clinical trial OR trial* OR "intervention study" OR RCT OR "randomized controlled trial" OR
23 24	165	"randomised controlled trial"
24 25 26	166	5. #2 AND #3
27 28	167	6. #1 AND #4 AND #5
29	168	The search syntax for other databases will be using similar approach as PubMed or using the
30 31	169	following merged search terms:
32	170	(Probiotic OR Lactobacillus OR Bifidobacterium OR Propionibacterium OR Saccharomyces OR
33	170	"Bacillus coagulans") AND (colon OR colorectal OR colonic OR rectal) AND (cancer OR
34	172	neopla* OR tumo* OR carcinoma OR malignan*) AND ("clinical trial" OR "intervention study"
35 36	172	OR "RCT" OR "randomised controlled trial" OR "randomized controlled trial")
37	1/5	
38	174	A systematic search of PubMed, Scopus, Web of Science, ProQuest and Wiley online library
39 40	175	was performed between 17 and 20 January 2020.
41 42	176	Selection process of included studies
42 43	177	The primary article screening will be carried out independently by two reviewers. Titles and
44	178	abstract of the studies will be screened independently, and the selected studies will be divided
45	179	into three groups: relevant, irrelevant and unsure. The studies which are categorized as irrelevant
46	180	by both reviewers will then be eliminated from the review. The full text of the remaining studies
47 48		
40 49	181 182	will be then reviewed by both reviewers using the eligibility criteria and studies that meet all the aritaria will be included. In any of diagrammer, the two reviewers will first meet to diagram their
50	182	criteria will be included. In case of discrepancy, the two reviewers will first meet to discuss their
51	183	choices and a final decision will be made. If there is any misunderstanding or conflict a third
52	184	opinion will be sought from the other reviewers and when an agreement is reached, a final
53 54	185	decision will be made.
54 55	186	
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58 59		6
60		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

187 Data extraction and Analysis

Studies meeting the inclusion criteria will be processed for data extraction. Two authors (DIJ and MAA) will independently screen title and abstract, and then full text. The data will be extracted and recorded in a consistent way using standardized data extraction form. The following data will be extracted: study year, author/s, study title, number of participants, stage of colorectal cancer, type of probiotic used, dosage of intervention, duration of intervention, control or placebo used, primary outcomes, secondary outcomes, conclusion and limitation.

13
 194 The search and study framework will be represented using PRISMA flow chart³⁰ and the
 195 numbers of all included and excluded studies will be reported and the reasons for exclusion of
 196 studies will be given.

17 18 197 Assessment of risk of bias of included studies

The risk of bias will be assessed according to the guidelines of the Cochrane Collaboration using ROB tool.³¹ this tool will be based on the following domains: random sequence generation, allocation concealment, adequacy of blinding for participants, blinding of outcome assessment, incomplete outcome data and selective reporting, and other sources of bias. RoB 2.0 will be used for risk of bias assessment of included study via RevMan version 5.3 software.³² Two reviewers independently will carry out the assessment and if there is any conflict, third opinion will be obtained from third partner.

29 205

31 206 Strategy for data synthesis

Initial screening of the relevant RCT studies showed that most of the outcomes of the included studies are not homogenous and cannot be pooled together, therefore meta-analysis most likely will not be carried out. Instead, a qualitative analysis will be performed to synthesize the studies included in the systematic review as well as a critical appraisal of the outcomes will be considered for all studies. However, after we complete the data extraction of all included studies if we find out that any of the outcomes is homogenous across some of the studies, then a meta-analysis of those selected outcomes will be carried out. The quality of all included studies will be assessed by using PRISMA checklist³⁰ to ensure that the included studies are of good quality and to ensure that there is no publication bias.

45 216 **Patients and public involvement**

There will be no need to involve patients or members of the general public in the design of this
systematic review, and no patients or member of the public will be contacted in order to
complete the systematic review.

51 220 Ethics and dissemination

Findings of this systematic review will be published in a peer-reviewed publication and will be
 presented at a professional conference. Because this is only a protocol, no ethical assessment is
 required.

2		
3 4	224	Author Contributions
5	225	DIJ and MAA contributed to the conception of the study. The systematic review protocol was
6	226	drafted by DIJ and was reviewed by MAA and AMA. The search strategy was developed by DIJ
7	227	and MAA and will be performed by DIJ and MAA, who will also independently screen the
8 9	228	potential studies, extract data from the included studies, assess the risk of bias and complete the
10	229	data synthesis. AMA and SH will arbitrate in cases of disagreement and ensure the absence of
11	229	errors. All authors reviewed approved the publication of the protocol.
12	250	enors. An autions reviewed approved the publication of the protocol.
13 14	231	Funding statement
15	232	This research received no specific grant from commercial or not-for-profit sectors. University of
16	233	Malaya will support the systematic review study with FRGS grant number FP103-2019A.
17 18		
19	234	Competing interests' statement: NONE
20 21	235	Word count: >2500 words
22	236	Data statement section: Not Applicable
23	•••	
24 25	237	
26	238	
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28	239	
29 30	240	
30 31	240	
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Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the PRISMA-Preporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

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32 33 -			Reporting Item	Page Number
34 35	Title			
36 37 38	Identification	<u>#1a</u>	Identify the report as a protocol of a systematic review	Page 1
39 40 41 42	Update	<u>#1b</u>	If the protocol is for an update of a previous systematic review, identify as such	N/A
13	Registration			
14 15 16 17 18		<u>#2</u>	If registered, provide the name of the registry (such as PROSPERO) and registration number	Under review by PROSPERO
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56 57 58 59	Contribution	<u>#3b</u>	Describe contributions of protocol authors and identify the guarantor of the review r review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	Page 7
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1 2	Amendments					
2 3 4 5 6 7 8 9		<u>#4</u>	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A		
10 11	Support					
12 13 14 15	Sources	<u>#5a</u>	Indicate sources of financial or other support for the review	Page 7		
16 17	Sponsor	<u>#5b</u>	Provide name for the review funder and / or sponsor	N/A		
18 19 20 21	Role of sponsor or funder	<u>#5c</u>	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	N/A		
22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58	Introduction					
	Rationale	<u>#6</u>	Describe the rationale for the review in the context of what is already known	Page 3-4		
	Objectives	<u>#7</u>	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Page 4		
	Methods					
	Eligibility criteria	<u>#8</u>	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Page 4-5		
	Information sources	<u>#9</u>	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	Page 5		
	Search strategy	<u>#10</u>	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Page 5-6		
	Study records - data management	<u>#11a</u>	Describe the mechanism(s) that will be used to manage records and data throughout the review	Page 6		
59 60		For pee	er review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml			

1 2 3 4 5 6	Study records - selection process	<u>#11b</u>	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Page 6
7 8 9 10 11 12 13	Study records - data collection process	<u>#11c</u>	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Page 6
14 15 16 17 18 19	Data items	<u>#12</u>	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Page 6
20 21 22 23 24	Outcomes and prioritization	<u>#13</u>	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Page 5
25 26 27 28 29 30 31	Risk of bias in individual studies	<u>#14</u>	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	Page 6
32 33 34	Data synthesis	<u>#15a</u>	Describe criteria under which study data will be quantitatively synthesised	Page 6
 35 36 37 38 39 40 41 42 43 	Data synthesis	<u>#15b</u>	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	N/A
44 45 46 47	Data synthesis	<u>#15c</u>	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	N/A
48 49 50	Data synthesis	<u>#15d</u>	If quantitative synthesis is not appropriate, describe the type of summary planned	Page 7
51 52 53 54 55 56	Meta-bias(es)	<u>#16</u>	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	N/A
57 58 59 60	Confidence in cumulative	<u>#17</u> For pee	Describe how the strength of the body of evidence will be assessed (such as GRADE) r review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	N/A

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2 3 4	None The PRISMA-P checklist is distributed under the terms of the Creative Commons Attribution License CC-BY 4.0. This checklist can be completed online using <u>https://www.goodreports.org/</u> , a tool
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