



BMJ Open Mapping the available evidence on the impact of ingested live microbes on health: a scoping review protocol

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ABSTRACT

Introduction It has been hypothesised that the regular consumption of safe, live microbes confers health-promoting attributes, including the prevention of disease. To address this hypothesis, we propose a scoping review approach that will systematically assess the large corpus of relevant literature that is now available on this research topic. This article outlines a protocol for a scoping review of published studies on interventions with live microbes in non-patient populations across eight health categories. The scoping review aims to catalogue types of interventions, measured outcomes, dosages, effectiveness, as well as current research gaps.

Methods and analysis The scoping review will follow the six-staged protocol as proposed by Arksey and O'Malley and will include the following stages: defining the research questions (stage 1); defining the eligibility criteria and finalising search strategy (stage 2); selection of studies based on the eligibility criteria (stage 3); development of a data extraction framework and charting of data (stage 4); aggregation of results and summarisation of findings (stage 5); and the optional consultation with stakeholders (stage 6), which will not be performed.

Ethics and dissemination Since the scoping review synthesises information from existing literature, no separate ethical approval is required. The findings of the scoping review will be communicated for publication to an open-access, peer-reviewed scientific journal, presented at relevant conferences, and disseminated at future workshops with all relevant data and documents being available online through the Open Science Framework (<https://osf.io/kvhe7>).

INTRODUCTION

More than a century ago, Russian Nobel laureate Ilya Ilyich (Élie) Metchnikoff theorised that human health could be enhanced and senility delayed to increase the lifespan of human beings through manipulation of undesirable microbes in the gut through the consumption of host-beneficial bacteria found in yoghurt.¹ Metchnikoff's theories originated from his observations of an unusually large number of centenarians in the Balkan States and Russia, who were often from humble circumstances and led extremely

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The present protocol is for a scoping review, providing a pragmatic way to systematically assess the wide range of studies evaluating the effect of consumption of live microbes on preventive endpoints for several health categories.
- ⇒ The scoping review includes a broad gamut of live microbe interventions (probiotics and fermented foods/beverages) and health categories, thereby providing a comprehensive overview of the available body of literature and research gaps.
- ⇒ Only articles published in English and available in PubMed, Scopus and Cochrane databases were considered for inclusion in the scoping review.
- ⇒ A Jadad scoring system will be used for quality assessment of studies found eligible for inclusion; such assessments are usually beyond the purview of scoping reviews and more appropriate for other review formats such as systematic reviews and meta-analyses.
- ⇒ A lack of granularity in fermented food interventions compared to those involving probiotics, along with a considerable variation in several features (strains of microbes, microbial load, sensory traits, etc) even among the same fermented foods, can make it difficult to report certain aspects of such studies in a standardised manner.

simple lifestyles. Besides elements from their simple lifestyle, Metchnikoff attributed their longevity to the consumption of a yoghurt-like soured milk, which, according to him, allowed suppression of putrefactive colonic bacteria through the ingestion of lactic acid bacteria used to prepare these foods.¹ Over a century later, Metchnikoff's observations have found widespread scientific acceptance and are currently a subject of intense medical research and also the source of a multibillion dollar global industry.²

Metchnikoff's work is the basis for the probiotics industry, with probiotics being defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'.³ Microorganisms

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closely related to probiotics are often encountered in fermented foods, which are defined as 'foods made through desired microbial growth and enzymatic conversions of food components'.⁴ Microbes in fermented foods however may not have 'proven' health claims, that is, ones that have been accepted by regulatory bodies, and therefore cannot be termed probiotics. Several recent studies have demonstrated that probiotics (as dietary supplements) and fermented foods, which are the primary sources of ingested microbes in human populations, can positively modulate the gut microbiota to alleviate specific human diseases.^{3–6} Indeed, over the last decade, the human intestinal microbiota has emerged as an important factor associated with suboptimal health conditions with intestinal dysbiosis commonly observed in such conditions.^{4 7–9} Probiotics and fermentation-associated microbes (from fermented foods) have been shown to be capable of restoring such dysbiotic microbiota thereby facilitating disease alleviation among other health benefits.^{10–13} It has been postulated that a reduced exposure to fermentation-associated beneficial microbes due to consumption of predominantly processed foods in today's world may have led to a rise in chronic metabolic, immune and 'lifestyle' diseases, which are often associated with a dysbiotic microbiota and inflammation.^{7 14 15} Indeed, the 'old friends' hypothesis suggests that exposure to benign or commensal microbes in foods might be an important, necessary source of microbial stimuli for the immune system.⁷

The growing corpus of evidence from various studies and research associating ingestion of specific live microbes with health benefits has led to the hypothesis that regular consumption of safe, live microbes may confer health-promoting attributes contributing to prevention, mitigation or risk reduction for diseases.⁷ As an extension, this has raised the possibility for a recommended daily allowance or an adequate intake for live microbes. To do this, however, there is a need to assess and synthesise the currently available scientific evidence to understand the relationship between consumption of live microbes and concomitant health benefits. To address this, we propose performing a scoping review of the relevant available literature involving interventions with live microbes in 'non-patient' populations (ie, subjects without pre-existing, chronic or genetic conditions that predispose them to relevant disease conditions) for 'non-therapeutic' or 'preventative' endpoints (ie, outcomes relevant to or indicating reduction of disease incidence or improvement in disease risk factors/markers).

A scoping review approach represents an appropriate methodology for reviewing large bodies of literature, which allows researchers to produce an overview of the available research on the topic, to summarise results and to identify evidence gaps.¹⁶ The approach does not necessarily involve critical appraisal of studies as they may be heterogeneous in terms of experimental design, methodology and therefore, quality of results reported.¹⁷ Despite this limitation, a scoping review of the topic may be

valuable in at least two respects. First, the broader scope of the scoping review will allow the study to overcome the narrow foci of systematic reviews on similar topics and adopt a comprehensive approach to the topic. Indeed, according to Cochrane summaries, several systematic reviews and meta-analyses are available for the effect of probiotics on the treatment of diverse health conditions, but few reviews are available that focus on enhancing our understanding of the effects of fermented foods and probiotics on non-patient populations. Second, in tandem with growing research on gut microbiology, several systematic reviews on related topics representing a subset of gut microbiology research have been published and a synthesis of the growing evidence is necessary.^{18–20} In this article, we present the protocol that will inform the execution of this scoping review.

Methods and analysis

Protocol design

The scoping review will be carried out following the framework proposed by Arksey and O'Malley,¹⁷ and further developed by Levac *et al*²¹ and the Joanna Briggs Institute (JBI).²² Accordingly, our protocol will include at least five stages for the review process and will incorporate elements from previous studies.^{6 23–25} The five stages of the review process are as follows:

- Stage 1: identifying research questions.
- Stage 2: identification of relevant studies.
- Stage 3: selection of studies.
- Stage 4: charting of data.
- Stage 5: collecting, summarising and reporting of results.

Importantly, the original framework proposed by Arksey and O'Malley included an optional consultation exercise with key stakeholders (stage 6)¹⁷ to facilitate identification of additional references regarding potential studies that may be included and also to collect feedback concerning the findings of the scoping review exercise itself. This final stage of the scoping review exercise, although valuable, will not be executed for the present scoping review due to time and budget constraints.

Patient and public involvement

No patients were involved in the review protocol and are planned to be involved in the review process as it is based only on literature that is already published. The public was not involved in the design of the review protocol, and will not be involved in the conducting of the protocol, or reporting and dissemination of the work. No plans to involve public stakeholders after completion of the review are currently being pursued.

Stage 1: identifying research questions

Prior to the identification of research questions, an exploratory review of the available literature on the effect of live microbes on various health conditions facilitated the refinement of the scope of the present review protocol. This phase informed the decision, whereby a

Box 1 List of health categories included in the scoping review

Health categories

- ⇒ Antibiotic-associated diarrhoea/*Clostridium difficile*-associated diarrhoea.
- ⇒ Gastrointestinal health.
- ⇒ Urogenital health.
- ⇒ Weight management.
- ⇒ Cancer.
- ⇒ Cardiovascular health and metabolic syndrome.
- ⇒ Respiratory health.
- ⇒ Immunological health.

final list of health categories to be investigated in relation to their prevention was drawn up (box 1), along with refining of the population scope. On the basis of the preliminary exploratory review of available literature, the following research questions were identified:

1. What is the relationship between ingestion of live microbes and prevention/risk reduction of various health conditions in non-patient populations over 2 years of age?
2. What are the types of non-therapeutic interventions involving live microbes that are addressed in the literature for non-patient populations?
3. What are the measures used to assess health outcomes?
4. Is there a quantitative relationship between the ingestion of live microbes and prevention/risk reduction of various health conditions?
5. How effective are such interventions and what is the scale of risk reduction/prevention?
6. What is the evidence of effectiveness when interventions may be combined?
7. How is the gut microbiota influenced in non-therapeutic interventions with live microbes for various health conditions?
8. Are there certain populations and/or demographics that are under-represented in such research?
9. What are the limitations of non-therapeutic interventions involving live microbes in terms of study design and what manner of refinements may be necessary to facilitate optimal experimental design?

Stage 2: identification of relevant studies

In accordance with the framework proposed by Arksey and O'Malley, the second stage of the scoping review process will involve identification of criteria that will inform the selection of studies for inclusion in the review. These criteria will help guide the review search process by filtering out irrelevant sources; this is particularly useful when reviewing a broad spectrum of literature as scoping reviews are designed to do. Based on the initial exploratory research, the following eligibility criteria were agreed upon:

- Type of publication: journal articles.
- Time frame: 2000 CE onwards.

- Bibliographical source: PubMed, Cochrane and Scopus.
 - Language: English.
 - Study population: healthy individuals 2 years or older; body mass index (BMI) $\leq 30 \text{ kg/m}^2$.
 - Setting: non-therapeutic, risk reduction or preventive therapy. Randomised controlled trials, cross-over trials and observational studies such as cohort studies, case-control studies and cross-sectional studies are included.
 - Health categories: see box 1.
 - Interventions: probiotic microorganisms (granted "Generally Recognized as Safe" status by the US Food and Drug Administration, "Qualified Presumption of Safety" status by the European Food Safety Authority or equivalent) and fermented foods/beverages.
 - Measured outcomes: outcome measures will be tailored to different health categories such as waist circumference in weight management, lipid profiles in cardiovascular health and so on.
- Certain criteria for exclusion that were agreed upon are as follows:
- Type of publication: review articles, systematic reviews, meta-analyses, scoping reviews, evidence maps, rapid reviews, literature reviews, evidence syntheses, reviews of reviews, narrative reviews and critical reviews, conference abstracts, book reviews, commentaries or editorial articles.
 - Interventions: products of alcoholic fermentation or fermented foods that are known not to contain live microbes in their final form, such as sourdough, were excluded.
 - Population: individuals below 24 months of age, preterm infants, pregnant women and breastfeeding cohorts were excluded.
 - Study setting: studies looking into recurrence of a health condition, *in vitro* studies, animal studies or studies only reporting changes in the gut microbiota will not be included.

A search strategy as recommended in standard JBI systematic reviews was followed, with iterative refinement of search terms as researchers become more familiar with the relevant literature available.²⁶ Briefly, the search strategy combined subject headings (Medical Subject Headings (MeSH) terms) along with appropriate text words to build the search codes for each health condition. The search codes were structured as: (interventions)+(health conditions)+(study types). The search strategy was developed and iteratively improved by one author in consultation with the lead investigator, if necessary. An academic librarian was consulted and provided advice regarding the most appropriate MeSH terms for the search. Search strings developed for MEDLINE based on this initial exploratory phase can be found in online supplemental data 1. Relevant documents retrieved through this search were imported and managed using a reference manager tool. The study has been registered in

the Open Science Framework portal (OSF; <https://osf.io/kvhe7>).²⁷

Stage 3: selection of studies

The third stage of the scoping review, as informed by the framework proposed by Arksey and O'Malley, will identify studies for inclusion in the scoping review. Results from search runs will be consolidated and de-duplicated. This will be followed by title- and abstract-based screening of retrieved studies to exclude those that do not meet the eligibility criteria identified in the second stage of the review protocol. For those studies that fulfil the eligibility criteria, full-text articles will be retrieved. A subset of retrieved articles (~20%) will be screened by another team member to ensure that the eligibility criteria for inclusion in the review have been consistently applied. Titles and abstracts of articles for which the team member could not determine eligibility for inclusion will also be reviewed by the second team member. Disagreements regarding the

study eligibility of the sampled articles will be discussed between the two reviewers until a consensus is reached; otherwise arbitration by a third reviewer may also be sought if required. Reasons for exclusion of any full-text source will be recorded and reported. A date will be set after which no additional studies will be included to maintain project timelines. Relevant articles discovered thereafter will be attached in an appendix to the final report. The process of study selection is reported here in terms of the Preferred Reporting Items for Scoping Reviews flow chart (PRISMA-ScR),²⁸ and will be duly updated once the review process is completed (online supplemental data 2). Critical appraisal of selected studies, although not mandatory in scoping reviews, will be carried out using the Jadad scoring system.²⁹

Stage 4: charting of data

A data extraction framework was developed based on the preliminary scoping phase (table 1). This framework is

Table 1 Data extraction framework for studies selected after full-text screening for inclusion in the scoping review

Main category	Subcategory	Description
1. Authors		
2. Title		
3. Journal		
4. Year of publication		
5. Country of study		
6. Objectives of study		
7. Type of study		Specify the type of study (eg, randomised controlled trial, cohort study, etc)
8. Description of intervention	Type of intervention	Specify the type of intervention—probiotics or fermented food and what microbe
	Microbe status	Specify if the microbes are delivered in an active, freeze-dried, spore form or unspecified
	Intensity/dosage of live microbes	Specify the quantity of live microbes in the intervention
	Length of intervention	Specify the length of the intervention
9. Description of study population	Target population	Describe the population targeted in the study including number of participants
	By age	Specify the age group(s) covered in the study
	By sex	Specify distribution of study participants by sex
	By BMI* (pre-intervention)	Specify the BMI of the population under study
	By other characteristics	Specify if there are any other characteristics of interest for the specific population under study
10. Setting of intervention		Specify the setting of the intervention (such as community based, school based, etc)
11. Reported outcomes		Describe the intervention outcomes reported in the study. For example, for weight management, relevant outcomes would include reported changes in BMI and body fat composition
12. Effectiveness		Describe the results reported in the study
13. Impact type		Describe if the impact of the intervention was positive, negative or neutral with respect to the health condition
*BMI: body mass index.		

not final and may undergo further modifications as the review process nears completion; it will be duly updated. Currently, the data extraction framework includes 13 categories that will be used to assess the full-text review articles found to be fulfilling the eligibility criteria for inclusion (table 1).

The data extraction framework will involve collection of bibliographical information such as authors, title, journal and year of publication, along with detailed information on interventions, populations and outcomes. For each study, the microbial species, strain, dosage, fermented food type and duration for the intervention will be recorded, as applicable. In terms of population details for each study, the distribution of participants by age, sex and other relevant characteristics (eg, BMI) will be reported. Other additional details to be recorded in the data extraction framework include the type of study, settings of the study, the types of measured outcomes assessed (eg, rates of incidence, levels for cytokines, cholesterol, etc), results of the study (effectiveness) and the type of impact (positive, negative, neutral) from the intervention (table 1). It must be noted that outcome measures will be tailored to each health category where the most clinically relevant and well-established measures will be prioritised.

The data extraction framework will be tested by two team members on a subset of studies included in the last stage (~10%) in order to ensure that the framework is applied consistently. As mentioned above, the framework may be modified and revised to further include/exclude categories as data extraction proceeds. Uncertainties and disagreements arising in this pilot stage of data extraction will be discussed by the team and resolved through consultations. The same two team members will then independently chart the data for each included study, following the data extraction framework as designed. Inter-reviewer reliability in data extraction will be ensured by a sample of the included studies (~20%) being independently reviewed by each reviewer and then compared between them. Discrepancies in data extraction, if any, will be discussed between the two reviewers until a consensus is reached; arbitration of a third reviewer may also be sought, if necessary. Data extraction framework, aggregated data and other relevant information will be made available through the OSF.²⁷

Stage 5: collecting, summarising and reporting of results

Data from the data extraction framework will be analysed to provide information on the research/evidence available on interventions involving live microbes in non-patient populations with non-therapeutic endpoints. Patterns and trends will be presented in visual form with accompanying narrative summary. These can include mapping the geographical distribution of studies, range of interventions, methods used and measured outcomes recorded, among others. Results will also be presented in a thematic manner, for example, according to health categories. To this end, a template will be developed and applied for each thematic group. This will include a

tabular and accompanying narrative summary of information relevant to research questions such as dietary source of live microbes, type of live microbes and sample sizes, among others, extracted from included studies. Such an approach will not only allow us to understand the available evidence on the health benefits of consuming live microbes, but also facilitate identification of contradictory evidence and knowledge gaps for each health condition. Final conclusions and future recommendations will be drawn from the mapped evidence. Additionally, reporting will attempt to conform with the recent PAGER (Patterns, Advances, Gaps, Evidence for practice and Research recommendations) framework which attempts to standardise the reporting of results from scoping reviews.³⁰

ETHICS AND DISSEMINATION

As the scoping review involves synthesis of information from publicly available publications and studies, no ethical approval is required. For dissemination purposes, an article reporting the final results of the scoping review will be communicated for publication to an open-access scientific journal, as per the Transparency and Openness Promotion Guidelines. The final findings of the scoping review, along with the protocol, data extraction framework and other relevant documents, will additionally be made available through the OSF and presented at relevant conferences and symposia. We anticipate that the findings of the scoping review will provide a comprehensive overview of the evidence currently available for supporting the hypothesis that a general, routine consumption of live microbes may promote health benefits as well as indicate research gaps and controversial research. The scoping review will provide important information to policymakers, academics, industrialists and healthcare professionals interested in funding, planning and delivering evidence-based, effective interventions to improve human health through diet. Results may hence be also disseminated as part of future workshops with diet and nutrition professionals.

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