

Protocol for a scoping review of fecal microbiota transplantation from patients into animals

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Abstract

Microbiota studies have uncovered numerous associations between human gut microbes and health-related outcomes. However, since most of these correlations were observed in cross-sectional studies, the causal inference is limited. The causal contribution of microbiota can be evidenced through disease induction or exacerbation in animal models after fecal microbiota transplantation (FMT) from patients.

In this article we present a protocol for a scoping review on the subject of FMT from humans to animals. Besides assessing how the published studies were conducted, in that scoping review we aim to find out whether enough literature exists to conduct a systematic review of the evidence for microbiota participation in the pathophysiology of any human non-infectious disease or phenotypic trait. We will conduct searches on the Web of Science platform and databases: MEDLINE, Scopus, EMBASE. Citation chasing of included studies will be done. We will include studies assessing the effects of FMT collected from people with certain medical conditions on animals. Studies that recruited only healthy humans or used other animals as donors will be excluded. The results of this literature search will be tabulated and discussed. Moreover, we will provide a short list of human non-infectious diseases or traits with the highest number of FMT patient-to-animal studies.

Keywords: Etiology · human microbiota-associated animal model · human stool transfer

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Introduction

In the past decade, there has been an undeniable increase in the investigation of the structure and function of the human gut microbiota, the complex community of microorganisms that inhabit the intestinal tract. Microbiota studies have uncovered numerous associations between human gut microorganisms and both surrogate and hard outcomes. They are believed to result from dysbiosis that can be understood as a bloom of pathobionts, loss of either commensals or keystone taxa, shifts in the metabolic capacity of microbiota or loss of microbial diversity [1]. However, since the majority of these correlations were noted in cross-sectional studies comparing healthy people with those who were ill, one cannot reliably distinguish whether observed dysbiosis is a cause, consequence or an epiphenomenon of the disease [2-3].

It is believed that the answer to this question is achievable in observational studies through the adaptation of more sophisticated computational methods, commonly with longitudinal study design. However, a recent review of current statistical methods supported with a simulation study concluded that they may yield biased or misleading results due to inherent features of the microbiome data, which are zero-inflated, over-dispersed, high-dimensional, multi-collinear, multivariate and highly variable [4]. Others assess causality using data from microbiome genome-wide association studies (mGWAS) and utilize a bidirectional Mendelian randomization approach [5]. Unfortunately, the certainty of this evidence is also limited, this time because of the strong assumptions underlying Mendelian randomization [6-7]. If these assumptions are not met (e.g. due to genetic pleiotropy), the Mendelian randomization results might be not invalid. Direct evidence of a causal relationship can be obtained using experimental methods, mainly using animal models.

One such method is fecal microbiota transplantation (FMT) in which a unique microbial enterotype from healthy donors is transferred to prevent or treat diseases [8]. Since several randomized clinical trials confirmed the efficiency of FMT in the treatment of recurrent *Clostridioides difficile* infection (rCDI) [9], the causal contribution of dysbiosis to the pathophysiology of this infection has been proven. It is interpreted that FMT restores a healthy, diverse gut microbiota that protects against further episodes of rCDI. On the other hand, FMT can be used to transfer material from patients or animal models with a particular disease in order to pre-clinically induce/exacerbate it in gut dysbiosis-related disease animal models. Animal recipients (usually germ-free) of human-donated FMT are called human microbiota-associated (HMA) animal models [10-11]. Beyond proving dysbiotic microbiota contribution to a particular pathology, it is also possible, at least in some cases, to find specific pathobionts or protective microbes causally contributing to it [12].

Even though HMA animal models also have several limitations (discussed in [10-13]), they are considered currently the best model to both demonstrate causation and to elucidate mechanisms linking microbiota to the pathophysiology of human diseases. Unfortunately, there are no summaries of FMT-co-transplanted phenotypes from patients into animals nor guidelines on how to perform such studies. Specifically, no current systematic or scoping reviews on the topic were found in MEDLINE (via PubMed) or *JBIC Evidence Synthesis*. Without systematic identification of studies that performed FMT from patients to create HMA animal models, it is not only impossible to reliably assess evidence supporting gut dysbiosis participation in human diseases, but also it can lead to unnecessary repetition of research along with the unethical waste of animal lives.

Currently, only a similar issue is covered by the guidelines for reporting on animal-to-animal fecal transplantation (GRAFT) studies that are based on the systematic review of murine transplantation protocols (mice were both the donor and the recipient) [14]. Even though Walter et al. systematically searched for studies reporting human microbiota-associated (HMA) rodents, they focused on the methodological and analytical limitations of such studies rather than the scope of the research [10].

We attempt to overcome the limitations of the aforementioned studies by performing a scoping review of primary studies that will answer somewhat different questions (listed below) with a broader search strategy. This scoping review's goal will be to evaluate the breadth, methodology and characteristics of the literature on FMT from humans into animals. We hope that our findings will help provide a foundation for guidelines for conducting and reporting HMA animal studies, as well as identify HMA animal models with an adequate amount of literature to conduct a systematic review with meta-analysis.

Specific research questions

1. Which human non-infectious diseases (or traits) were tried to be transplanted with FMT into animal models?
 - For each disease/trait: Which outcomes were reported?
 - For each outcome group: Did the authors report that FMT affect it?
2. How were these FMTs performed?
 - What were donor characteristics and how was the material obtained?
 - How FMT was prepared and administered?
 - What was the control for FMT?
 - What were animal characteristics, preparation and housing conditions?
3. Is there sufficient literature to conduct a systematic review with the aim of evaluating the evidence for microbiota participation in the pathophysiology of any human non-infectious disease (or phenotypic trait)?

Eligibility criteria

FMT recipients

All animal species will be considered eligible FMT recipients. These species include, but are not limited to, laboratory animal species commonly used in biomedical research (e.g. mice, rats). We will exclude studies that recruit humans to receive FMT, as it is not within the scope of our review.

Intervention

Eligible interventions include FMTs via any route (e.g. oral, rectal), frequency, and method of administration. We will include only studies that collected the material used for FMT preparation from people with certain medical conditions (e.g. colorectal cancer, hypertension) or traits (e.g. lack of response to immunotherapy). Studies that recruited only apparently healthy humans or used other animals as donors will not be eligible for the scoping review.

Control

All types of controls (e.g. vehicle solution, heat-killed FMT, FMT from healthy donor group), as well as not using control intervention, will be eligible.

Outcomes

Since we are interested in the scope of the literature, all outcomes reflecting changes in the health of FMT recipients (i.e. animals receiving FMT) will be eligible. These outcomes include, but are not limited to, the following groups: behavioral (e.g. distance traveled in open field testing), cardiovascular (e.g. left ventricular ejection fraction) and immunological (e.g. serum interleukin 17 level). Studies reporting uptake of donor microbiome profile as the only outcome of the FMT recipients (e.g. stability of human microbiome in the animal gastrointestinal tract) will be excluded because they will not answer our research questions.

Sources

This scoping review will consider both experimental and quasi-experimental study designs including randomized controlled trials, non-randomized controlled trials, before and after studies and interrupted time-series studies. We do not plan to include and analyze secondary research (e.g. systematic or narrative reviews).

Material and methods

The proposed scoping review will be conducted in accordance with the JBI methodology for scoping reviews [15-16].

Search strategy

An initial set of articles on the topic was retrieved from the study conducted by Walter et al. [10]. A full search strategy for the MEDLINE on the Ovid platform (see [Supplementary materials, Table S1](#)) was developed based on the following elements: (1) the text words contained in the titles and abstracts of relevant articles, (2) synonyms found in the MeSH thesaurus and during manual searches, (3) FMT synonyms identified by Green et al. [17], (4) a laboratory animals search filter developed by van der Mierden et al. [18]. For each included database or platform the search strategy, including all the chosen keywords and index terms, will be adjusted.

Studies published in any language will be included. The databases will be searched from the beginning until the present day.

The databases to be searched include MEDLINE (via Ovid), Scopus, EMBASE and the Web of Science platform. An additional search of the grey literature will be limited to the Open Dissertations database (via EBSCO). CitationChaser will be used to perform both backward and forward citation chasing of included studies [19].

Study selection

Following the search, all identified records will be collated and deduplicated. Following a pilot test, two independent reviewers will screen the titles and abstracts to exclude clearly irrelevant articles using the Rayyan QCRI reference manager web application [20]. Researchers will be blinded to each other's decisions. Then, all chosen potentially eligible articles will be downloaded in full-text versions and thoroughly evaluated by two reviewers using the inclusion/exclusion criteria. We will record the reason for excluding each full-text record. Because we are interested in the details of methodologies and the scope of the available evidence, conference abstracts will be classified as "awaiting assessment" studies. Any disagreements that arise between the reviewers at each stage of the selection process will be resolved after unblinding through discussion or by an additional experienced reviewer (JR or DS). The results of the search and the study selection process will be illustrated using a PRISMA 2020 flow diagram and described in the final scoping review [21].

Data Extraction

Two reviewers will independently extract data from papers included in the scoping review using a self-developed data extraction tool. We will extract specific details about the animals (e.g. species, strain, age), their preparation and housing (e.g. antibiotic/laxative pre-treatment, number of animals in cage), donor characteristics (e.g. sex, disease/trait explored), FMT preparation and storage, FMT dose and administration and the outcomes assessed. A draft extraction form is provided

(see [Supplementary Table S2](#)). As data is extracted from each included article, the draft data extraction tool will be adjusted as necessary. Modifications will be described in the scoping review. Any disagreements that arise between the reviewers will be resolved through discussion or with the help of an additional reviewer. In the case of studies awaiting assessment, we will extract the following information: (1) disease/phenotypic trait of FMT donors, (2) species and strains of FMT recipients.

Data Analysis and Presentation

Extracted human non-infectious diseases/traits that were tried to be transplanted with FMT into animal models will be clustered according to the main affected system of the human body (cardiovascular, digestive, endocrine system and metabolic diseases, integumentary, immune and lymphatic, musculoskeletal, nervous, reproductive, respiratory, urinary). We will perform clustering of the assessed outcomes during data extraction (see [Supplementary Table S2](#), rows from “5. Assessed outcomes” for proposed clusters). According to the scoping review methodology [15], the analysis of the extracted data will be limited to the basic descriptive analysis (e.g. frequency counts of animal models). Basic characteristics of studies “awaiting assessment” will be presented in a table and they will not be included in the formal statistical analyses.

To answer the first research question (“Which human non-infectious diseases or traits were tried to be transplanted with FMT into animal models?”) and its sub-questions, we plan to tabulate the data on diseases/traits as rows (grouped by the system of the human body) and outcomes clusters as columns. Additionally, the basic descriptive analysis will be narratively presented.

To answer the second research question (“How were these FMTs performed?”) and its sub-questions, we plan to present descriptive statistics in a table and discuss them in the main body of the manuscript.

To answer the third research question (“Is there sufficient literature to conduct a systematic review with the aim of evaluating the evidence for microbiota participation in the pathophysiology of any human non-infectious disease (or phenotypic trait)?”), we will provide a short list of human non-infectious diseases or traits with the highest number of FMT patient-to-animal studies. With this list, researchers will be able to select topics of interest with the assurance of finding an adequate number of relevant studies for inclusion in future systematic reviews. We will mention all the issues that can be systematically reviewed while avoiding “empty” reviews (HMA animal models with at least one study) [22].

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Conflicts of interest

There is no conflict of interest in this project.

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