BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-052021
Article Type:	Protocol
Date Submitted by the Author:	03-Apr-2021
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Keywords:	Rheumatology < INTERNAL MEDICINE, Immunology < NATURAL SCIENCE DISCIPLINES, Microbiology < NATURAL SCIENCE DISCIPLINES

Gut Microbial Dysbiosis in the Rheumatoid Arthritis: A Systematic Review Protocol of Case-Control Studies

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Abstract

Introduction: Rheumatoid arthritis has a huge social impact due to the relatively high prevalence, irreversible joint damage and systemic complications. The gut microbiota plays an important role in the pathogenesis and progression of rheumatoid arthritis by directly or indirectly regulating the host immune system. Restoring intestinal homeostasis by altering the microbiota is an attractive strategy for the prevention and treatment of rheumatoid arthritis. However, the signature features of microbial dysbiosis in rheumatoid arthritis are still controversial. This review will clarify the characteristics of gut microbiome changes, hoping to provide new ideas for further understanding of the pathogenesis of rheumatoid arthritis.

Methods and analysis: We will include case-control studies which focus on the gut microbial dysbiosis in the rheumatoid arthritis as the primary outcome. Four databases

(including PubMed, EMBASE, Web of Science and Cochrane Library) have been searched and grey literature will also be systematically searched for. Eligible studies will be screened independently by two reviewers according to the inclusion criteria. The Newcastle-Ottawa Quality Assessment Scale will be used to assess the quality of the included studies. Data will be extracted, and meta-analyses will be performed within the gut microbial dysbiosis in the rheumatoid arthritis. The quality of evidence will be assessed by the Grading of Recommendations Assessment, Development, and Evaluation framework.

Ethics and dissemination: Ethical approval is unnecessary as this review does not address the data and privacy of patients' individuals. The results will be published in a peer-reviewed scientific journal and conference presentations.

PROSPERO registration number: CRD42021225229

Strengths and limitations of this study

This review will elucidate the characteristics of gut dysbiosis in patients with rheumatoid arthritis.

The findings of this study will provide a scientific basis for exploring the biomolecular link between the gut microbiota and the pathogenesis of rheumatoid arthritis.

Data pooled may be heterogeneous between studies due to gender, age, diet, medication, and specimen measurement methods.

Some studies published in non-English languages may be missed.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disease characterized by persistent synovitis, inflammatory and autoantibody changes[1]. The prevalence of RA is approximately 1% worldwide and 1.02% in China, with a high prevalence in women, 2-3 times higher than in men [2 3]. Delays in diagnosis and treatment are associated with worse outcomes, including irreversible joint destruction, disability and disease-related non-articular outcomes such as reduced life span[4 5]. In China, 77.6% of RA patients suffer from disability, moderate and severe disabilities account for about 39%, which seriously affected the quality of life[6]. With the deterioration of RA, the disease cost of patients increases sharply, which leads to a heavy social and economic burden on individuals and the country [7-9].

RA is an ancient disease with a complex pathogenesis and currently incurable disease [10]. European Association of Anti-Rheumatology Annual (EULAR) and American College of Rheumatology (ACR) recommend that the purpose of RA treatment should be to enable each patient to achieve the goal of continuous remission or low disease activity [11]. However, it has greatly limited the effectiveness of treatment due to unknown etiology, drug insensitivity, adverse effects, and massive medical costs, which make the condition of a large number of patients unable to be effectively alleviated [1 11-15]. Genetic, environmental and autoimmune factors are considered to play an important role in RA [16]. The gut microbiota maintains intestinal mucosal immune function and the integrity of the intestinal mucosal structure and is

considered an important environmental factor in the development of RA [17]. Almost all studies on autoimmune rheumatic diseases show abnormal microbial community structure (i.e. dysbiosis) [18]. Dysbiosis not only affects the pro-inflammatory and anti-inflammatory process of intestinal mucosa, but also affects the distal joint through the intestinal-joint axis [19-21]. It is very important to reduce the occurrence of RA, delay joint injury and avoid disability, through the improvement of intestinal flora imbalance.

The studies have found dysbiosis in RA patients as well as in high-risk individuals, indicating that the imbalance of intestinal flora has occurred before the onset of RA [17 22]. Dysbiosis has been involved in the pathogenesis of RA in the decade before its diagnosis [23]. The intestinal flora imbalance also appeared in the peak and relapse stage of RA [24]. The dysregulation of intestinal flora is related to the inflammatory response and disease activity of RA, which can be partially recovered by effective treatment [25-27]. The results of animal experiments suggest that interventions targeting intestinal microbiota may have the potential to prevent RA in the preclinical stage [28]. Intestinal flora has become a new therapeutic target, which plays an important role in the onset and progression of RA [29 30] .

There were significant differences in microbial diversity, species and function of RA intestinal flora. The abundance of Prevotella increased in patients with early RA, which had a negative impact on the development and prognosis of RA [17 31-34]. However, it has been reported that the abundance of Prevotella did not significantly change in RA patients [35]. Moreover, P. copri and P. histicola of Prevotella have

different effects on RA [17]. Bacteroidetes were enriched in female patients with RA, while Actinomycetes and Collinsella were enriched in healthy subjects [36]. However, the abundance of Bacteroides and Bifidobacterium was found to be reduced in RA patients and animal experiments [37 38]. Thus, the results of the study on intestinal flora were heterogeneous in RA patients. Through a quantitative review of the existing literature, the changes of RA intestinal flora can be understood more clearly and comprehensively. However, there have been no systematic reviews and meta-analyses on the characteristic changes of intestinal microbiota in RA to date. The purpose of this study will be to systematically review the case-control studies on the gut microbiota of RA, and use meta-analysis to quantitatively synthesize the results of the studies, so as to identify the biomarker of dysbiosis.

OBJECTIVE

This systematic review attempts to investigate the gut microbiota profiles of RA patients by synthesizing available the case-control trains to elucidate the biomarkers of dysbiosis with this disorder.

METHODS

Study design

We plan to conduct a systematic review according to the Cochrane Handbook for Systematic Reviews of Interventions Version 6.1[39], Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA)[40], and PRISMA-Protocols (PRISMA-P) 2015 [41], as well as the Newcastle-Ottawa Quality Assessment Scale (NOS)[42]. The PRISMA-P 2015 checklist is shown in Table 1. This protocol has been

registered at PROSPERO (registration number: CRD42021225229).

Eligibility criteria

The studies, written in English as eligible, will be selected and screened based on PICOS steps (Population, Interventions, Comparator, Outcomes, and Study design). In this systematic review, PICOS will be scientifically modified by substituting the item "Intervention" for "Investigation". The data items will be extracted as following:

Types of participants (P)

The population of interest of the eligible studies should be adults (≥18 years old) with met the diagnostic criteria(the ACR/EULAR 2010) for RA[43] or established RA (1987 classification criteria)[44] in the experimental group， the control group is a healthy population.

Type of Investigation (I)

Trials were applied to assess the gastrointestinal microbiota. Quantitative synthesis of gut microbiota in fecal samples was performed by using metagenomic shotgun sequencing, 16s rRNA sequencing techniques and/or real time polymerase chain reaction (rt-PCR).

Comparison (C)

All the following controls will be considered as eligible: healthy population or persons at high risk of RA.

Type of outcomes (O)

The main results will be taken into account: the composition of intestinal microbiome, changes in the gut microbiota diversity (alpha-diversity, beta-diversity), the relative

abundance of opportunistic pathogens and beneficial commensal bacteria. Additional outcome measures will be considered: faecal short chain fatty acids (SCFA) concentrations, and correlations between clinical, pathological parameters and relative abundance of microbial species.

Type of studies (S)

We will only include studies with the design of case-control studies. The original peer-reviewed articles written in English are considered. The publication types, such as animal studies, reviews, case reports and the full text unachieved will be excluded from the qualitative and quantitative synthesis.

Data sources and search strategies

The search will conducted using the databases EMBASE, PubMed, Web of Science, and Cochrane Library in English language published up to September 2020. After reading a number of documents, a search strategy combining medical subject terms (MeSH) and free words was developed: ("Arthritis, Rheumatoid " OR Rheumatoid arthritis OR RA) AND ("Gastrointestinal Microbiome " OR Gastrointestinal Microbiomes OR Microbiome, Gastrointestinal OR Gut Microbiome OR Gut Microbiomes OR Microbiome, Gut). In order to prevent the omission of the article, two researchers (DWW and XTP) will search the above database independently. Using the snowball method, we manually search for all references contained in the article.

Screening procedures of eligible studies

Once the search is complete, the literature will be managed using EndNote X9

（Clarivate Analytics （US） LLC）. Duplicates will be identified and deleted according to Literature title. Then, the titles and abstracts of the literature will be screened independently by two reviewers (XTP and YFL) according to the inclusion criteria. Retrieval of the full text will be based on the eligible of titles and abstracts, and the literature meeting all the inclusion criteria will be independently assessed. In case of disagreement, a third reviewer (ZLS) will be consulted. To measure interrater agreement, the Kappa coefficients will be both calculated for the processes of titles/ abstract selection and full-text screening. The criteria for judging the scope of the agreement between the evaluators are as follows: 0.00–0.20= slight agreement, 0.21–0.40= fair, 0.41–0.60= moderate, 0.61–0.80=substantial, and 0.81–1.00=almost perfect agreement[45]. The plan of study screening and selection is available in Figure 1.

Assessment of risk of bias

The quality of the included studies will be assessed using NOS [42]. It is a tool mainly used to evaluate the quality of case-control and cohort studies. The parameters considered under each category are: ① selection: case definition, representativeness of the cases, selection of controls and definition of controls; ② comparability: comparability of cases and controls on the basis of the design or analysis; ③ exposure: ascertainment of exposure, same method of ascertainment for cases and controls, non-response rate. There are 1 to 2 stars in each category, with a maximum of 9 stars for all. The number of stars is proportional to the mass of the study. The number of stars is directly proportional to the quality of the study. The standard of

high quality will be NOS score ≥ 7 stars.

To ensure consistency in assessments, the two reviewers (HXG and HZ) will independently evaluate the eligible literature according to NOS and will be summarized in a table. If disagreements arise in the review, they will be resolved by the third reviewer (ZLS) in collaboration with the team to reach consensus.

Data extraction

Data from each eligible article will be extracted and compiled using a standardized excel sheet. Items required for extraction will be obtained the PICOS steps. The following data will be extracted for eligible studies: first author's surname, year of publication, country, classification criteria for RA, number of cases and controls, age and sex, disease extent, antibody positive of RA, 28-joint disease activity score, medication, assessment methods of fecal microbiota, SCFA concentrations, alterations in gut microbial diversity. To ensure the accuracy of the extracted data, we will randomly select two eligible literatures, which will be independently extracted by two reviewers (FQC and RZ). Kappa will be applied to compare the consistency of data extraction from the two literatures by the two reviewers. If there is an almost perfect agreement between the two reviewers (Kappa value ≥80%), the remaining literature will extracted by one of the two reviewers.

Data synthesis and analysis

The included literature will reported the percentage of gut bacteria, also known as relative abundance, in RA patients and controls. If sufficient data will be available to calculate a pooled effect estimate in eligible studies, we will consider conducting a

meta-analysis. We will standardize all extracted data. In turn the relative abundance and standard error from each study will be used to obtain the total percentage of bacteria of different phyla and genera in RA patients and controls. To clarify the diversity changes in bacteria between RA and healthy people, we will calculate their percentages for the phyla and genera of each differential bacterium between the two groups. A random effects meta-analysis will be performed using Review Manager 5.3 software (the Cochrane Collaboration, Copenhagen, Denmark)[46]. We will use forest plots to visualize the results. We will assess heterogeneity between studies using the Higgins I^2 statistic. In relative terms, I^2 values are proportional to heterogeneity: I^2 values of 0-30% means minimal heterogeneity, 31-50% means moderate heterogeneity, and > 50%means substantial heterogeneity [47]. If meta-analysis is not feasible, we will conduct narrative synthesis to summarize the relevant evidence between RA and gut dysbiosis.

Assessment of publication bias

We will apply Begg's funnel plot and Egger's test to assess publication bias [48]. Publication bias will be considered if there is an asymmetrically shaped Begg's funnel plot or Egger's test p-value < 0.10.

Assessment of evidence quality

We will conduct an assessment of the quality of evidence by applying the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework [47]. Five domains will be assessed by two reviewers (YF and RZ), which is limitations of design, inconsistency, indirectness, imprecision and publication bias. The GRADE

classifies the quality of evidence into 4 levels, high, moderate, low, and very low. Disagreement on the assessment will be resolved by a third reviewer (ZLS).The GRADE Evidence Profiles will be generated using GRADEpro GDT (https://grade pro. org/).

ETHICS AND DISSEMINATION

Ethical approval is not necessary because the systematic review does not deal with the patient's personal data and privacy. The findings will be published in a peer-reviewed publication and conference presentations. This systematic review will be included as a chapter in the primary author's (DWW) PhD degree research thesis.

PATIENT AND PUBLIC INVOLVEMENT

No patient or public involved.

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Author Statement DWW and XTP drafted the manuscript and contributed equally to this manuscript as joint first authors. YFL provided the materials. HXG and HZ collected and assembled the data. FQC, RZ and YF analyzed and interpreted the data. ZLS conceived the study and critically revised the draft. All authors assisted in

manuscript editing and approved its contents.

Funding This work was supported by National Natural Science Foundation of China [81774383], Philosophy and Social Science Research of Jiangsu Higher Education Institutions [2020SJA0335], Post-graduate Research & Practice Innovation Program of Jiangsu Province [KYCX20_1449, KYCX20_1616], Qinglan Project Foundation of Jiangsu Province, Nursing Professional Innovation Practice and Teaching Team Open Fund of Nanjing University of Chinese Medicine [NZYHLXPPQL2019-26].

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Word Count: 1,989 words.

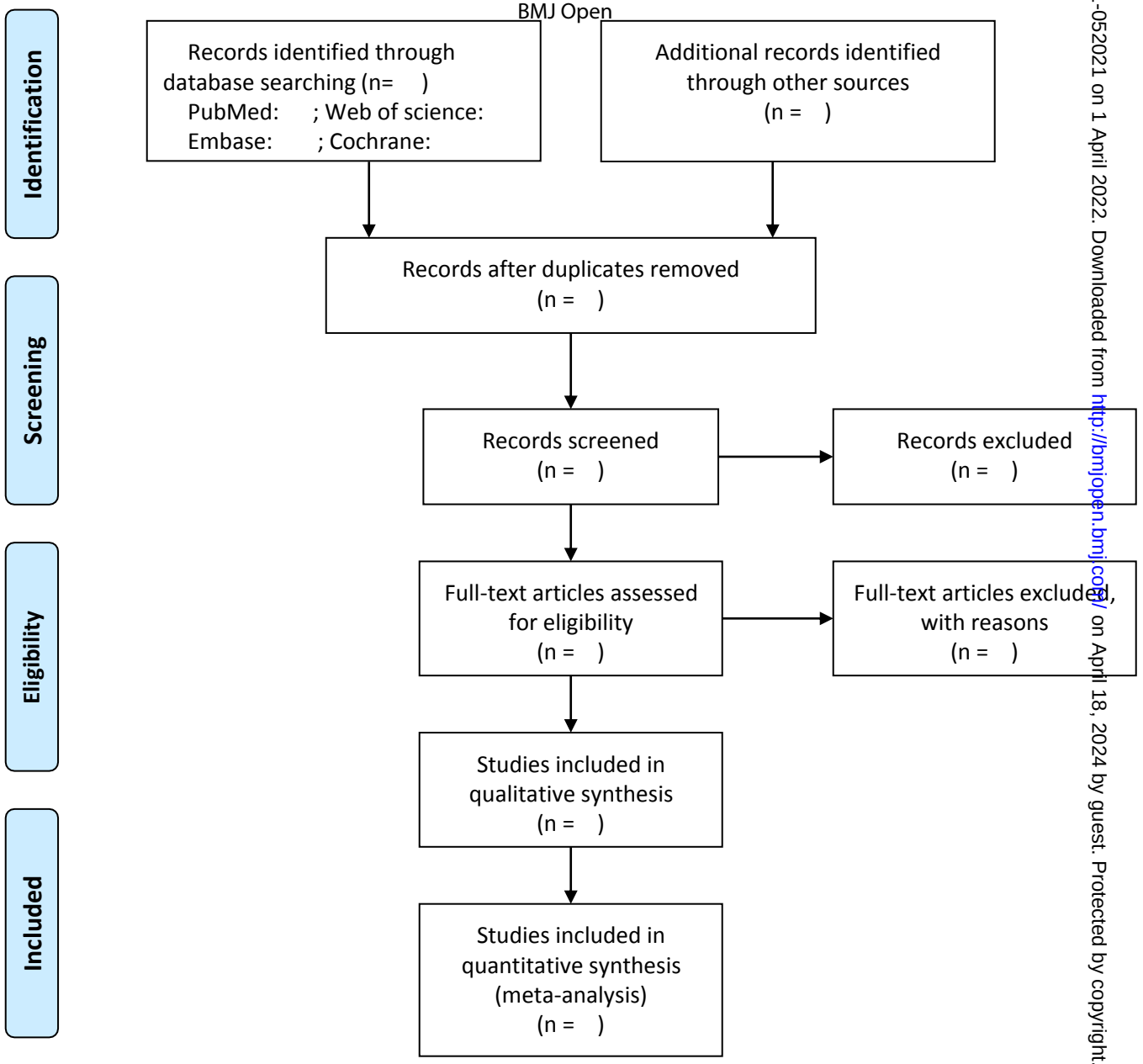

Figure 1 Plan of study screening and selection process

Table 1 PRISMA-P 2015 checklist

Section and topic	Item No	Checklist item	Reported on Page #
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	14-15
Amendments	4	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
Support:			
Sources	5a	Indicate sources of financial or other support for the review	15
Sponsor	5b	Provide name for the review funder and/or sponsor	N/A
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	N/A
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3-5
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	5
METHODS			
Eligibility criteria	8	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	6-7
Information sources	9	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	7
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	7
Study records:			

Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8
Selection process	11b	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)	8
Data collection process	11c	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	8
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	9
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	9
Risk of bias in individual studies	14	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	8-9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	9-10
	15b	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 I^2, Kendall's τ)	10
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	10
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	10
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	10
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	10-11

BMJ Open

Gut Microbial Dysbiosis in the Rheumatoid Arthritis: A Systematic Review Protocol of Case-Control Studies

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-052021.R1
Article Type:	Protocol
Date Submitted by the Author:	14-Dec-2021
Complete List of Authors:	Wang, Dan-Wen; Nanjing University of Chinese Medicine, Pang, Xiang-tian; Nanjing University of Chinese Medicine Zhang, Heng; Nanjing University of Chinese Medicine Gao, Hai-xia; Nanjing University of Chinese Medicine Leng, Yu-fei; Shanghai Jiao Tong University School of Medicine, Animal Surgery Laboratory Chen, Feng-qin; Nanjing University of Chinese Medicine Zhang, Rui; Nanjing University of Chinese Medicine Feng, Yun; Nanjing University of Chinese Medicine Sun, Zhi-ling; Nanjing University of Chinese Medicine
Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Rheumatology, Immunology (including allergy)
Keywords:	Rheumatology < INTERNAL MEDICINE, Immunology < NATURAL SCIENCE DISCIPLINES, Microbiology < NATURAL SCIENCE DISCIPLINES

Gut Microbial Dysbiosis in Rheumatoid Arthritis: A Systematic Review Protocol of Case-Control Studies

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Abstract

Introduction: Rheumatoid arthritis (RA) has a huge societal impact due to the high prevalence, irreversible joint damage and systemic complications. Gut microbiota plays an important role in the pathogenesis and progression of RA by regulating the host immune system. Restoring intestinal homeostasis by altering the microbiota could be an attractive strategy for the prevention and treatment of RA. However, the signature features of microbial dysbiosis in RA are still controversial. Therefore, we aim to elucidate the characteristic change in the diversity and composition of gut microbiota in RA.

Methods and analysis: We will systematically search through PubMed, EMBASE, Web of Science and Cochrane Library, as well as dissertations and conference proceedings. The reference lists of all included studies will be also reviewed to retrieve additional relevant studies. The case-control studies that reported either the relative abundance of bacteria at the phylum or genus level or at least one of the alpha-, beta-diversity indexes in both RA and health controls will be included. Eligible studies will be screened independently by two reviewers according to the inclusion criteria. The Newcastle-Ottawa Quality Assessment Scale will be used to assess the quality of the included studies. Data extraction, qualitative and quantitative analysis will be performed within the gut microbial dysbiosis in RA. The expected outcomes will be the specific changes in composition and diversity of the gut microbiota in patients with RA. The quality of evidence will be assessed by the Grading of Recommendations Assessment, Development, and Evaluation framework.

Ethics and dissemination: Ethical approval is unnecessary as this review does not address the data and privacy of patients. The results will be published in a peer-reviewed scientific journal and conference presentations.

PROSPERO registration number: CRD42021225229

Strengths and limitations of this study

This systematic review will identify the characteristic changes in the composition and diversity of gut microbiota in patients with RA, a significant but controversial clinical

issue.

The percentage and relative abundance of phyla or genus levels in the gut microbiota will be used in this analysis to avoid potential variation due to different detection methods of the microbiome in the included studies.

The Web Plot Digitizer will be used to digitize and extract data from graphs and plots may lead to biased results.

This systematic review will only include studies written in English, which may limit available data or result in language bias.

INTRODUCTION

RA is a chronic disease characterized by persistent synovitis, inflammatory and autoantibody changes [1]. The prevalence of RA is about 1% globally, and 1.02% in China [2]. The prevalence of RA in women is 2-3 times higher than that in men [3]. Delays in diagnosis and treatment are associated with worse outcomes, including irreversible joint destruction, disability and disease-related non-articular outcomes such as reduced life span [4 5]. In China, 77.6% of RA patients had disabilities, among which moderate and severe disabilities accounted for about 39%, seriously affecting the quality of life of patients [6]. The gradual deterioration of RA leads to a sharp increase in the cost of the disease, which imposes a heavy societal and economic burden on individuals and the country [7-9].

RA is an ancient disease with a complex pathogenesis and is currently an incurable disease [10]**.** European League Against Rheumatism (EULAR) and American College of

Rheumatology (ACR) recommend that the purpose of RA treatment should be to enable each patient to achieve the goal of continuous remission or low disease activity [11]. The prognosis of RA has improved in recent decades with advances in diagnosis and treatment. However, as the etiology and pathogenesis of RA are not fully understood, the therapeutic effect is greatly reduced, which seriously hinders the effective remission of RA patients [1 11-15]. Therefore, it is particularly important to explore the etiology and pathogenesis of RA.

Environmental factors are considered to play an important role in RA [16]. The gut microbiota is considered an important environmental factor in the development of RA [17]. Almost all studies on autoimmune rheumatic diseases show abnormal microbial community structure (i.e. dysbiosis) [18]. Dysbiosis not only affects the pro-inflammatory and anti-inflammatory process of the intestinal mucosa, but also affects the distal joint through the intestinal-joint axis [19-21]. The studies have found dysbiosis in both RA patients and high-risk individuals, indicating that the imbalance of intestinal flora has occurred before the onset of RA [17 22]. Dysbiosis has been involved in the pathogenesis of RA in the decade before its diagnosis [23]. The intestinal flora imbalance also appeared in the initial peak and relapse stage of RA [24]. Dysbiosis is related to the inflammatory response and disease activity of RA, which can be partially recovered by effective treatment [25-27]. As a first-line treatment for RA, methotrexate (MTX) may act in part by modulating the human gut microbiota [27]. The results of animal experiments suggest that interventions targeting intestinal microbiota may have the potential to prevent RA in the preclinical stage [28]. Probiotics

supplementation as adjunctive therapy improves the inflammatory state of RA in human and animal studies [29-32]. Therefore, gut microbiota plays an important role in the development of RA, and may be a new therapeutic target [33 34]. Gut microbiome studies of RA are essential to elucidate etiology and pathophysiological mechanisms and to develop potential therapeutic strategies. Regulating the gut microbiota to slow the progression of the disease, especially in the preclinical phase of RA, may be a promising approach for the treatment of RA in the future [35 36].

Although numerous studies have shown that dysbiosis of the gut microbiome is a key hallmark of RA, the distinct composition of the gut microbiome in RA patients remains controversial. The abundance of *Prevotella* increased in patients with early RA, which hurt the development and prognosis of RA [17 37-40]. However, it has been reported that the abundance of *Prevotella* did not significantly change in RA patients [41]. Moreover, *P. copri* and *P. histicola* of *Prevotella* have different effects on RA [17]. *Bacteroidetes* were enriched in female patients with RA, while *Actinomycetes* and *Collinsella* were enriched in healthy subjects [41]. However, the abundance of *Bacteroides* and *Bifidobacterium* was found to be reduced in RA patients and animal experiments [42 43]. It follows that the results of studies on the gut microbiota of RA patients are contradictory. The identification of specific microbial profiles and patterns that may contribute to the pathogenesis of RA remains a major challenge due to the inconsistent results of studies on the gut microbiota. The conflicting results may stem from inter-study batch effects, such as various biological factors influencing gut microbiome composition, different data processing and analysis methods [44 45].

Through a quantitative review of the existing literature, the changes of RA gut microbiota can be understood more clearly and comprehensively. Recently, several meta-analyses of gut microbiota have identified specific microbial biomarkers associated with disease [46-51]. However, there has been no systematic review and meta-analysis focusing on the characteristic dysbiosis of gut microbiota in RA to date. Therefore, we will perform a systematic review and meta-analysis to identify characteristic alterations in the gut microbiota of RA patients.

OBJECTIVE

The purpose of this protocol is to outline a systematic review and meta-analysis, which evaluates the changes in the diversity of gut microbiota and the relative abundance of bacterial phyla or genera in patients with RA.

METHODS

Study design

We plan to conduct a systematic review according to the Cochrane Handbook for Systematic Reviews of Interventions Version 6.1 [52], Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA)[53], and PRISMA-Protocols (PRISMA-P) 2015 [54], as well as the Newcastle-Ottawa Quality Assessment Scale (NOS)[55]. The PRISMA-P 2015 checklist is shown in Table 1. This protocol has been registered at PROSPERO (registration number: CRD42021225229).

Eligibility criteria

The studies, written in English as eligible, will be selected and screened based on PECOS steps (Population, Exposure, Comparator, Outcomes, and Study design) [56 57].

The data items will be extracted as following:

Types of participants (P)

The population of interest of the eligible studies should be adults (≥18 years old) with met the diagnostic criteria(the ACR/EULAR 2010) for RA [58] or established RA (1987 classification criteria) [59] in the experimental group， the control group is a healthy population.

Type of exposure (E)

Trials were applied to assess the gut microbiota. Quantitative synthesis of microbiota in fecal samples was performed by using metagenomic shotgun sequencing, 16s rRNA sequencing techniques and/or real-time polymerase chain reaction (rt-PCR).

Comparison (C)

Only healthy adults will be considered eligible for the control group.

Type of outcomes (O)

The primary outcome of the study will be the composition of the gut microbiome and the relative abundance of bacteria in RA. The secondary outcomes will be considered: changes in the gut microbiota diversity (alpha-diversity, beta-diversity), the effects of different gender and region on the relative abundance of gut microbiota.

Type of studies (S)

We will only include studies with the case-control design, written in English and published in the original peer-reviewed journals. The animal studies, reviews, case

reports, and the full text unachieved will be excluded from the qualitative and quantitative synthesis.

Data sources and search strategies

We conduct the search using the databases Embase, PubMed, Web of Science, and Cochrane Library in the English language published up to September 2020. After reading several documents, a search strategy combining medical subject terms (MeSH) and free words was developed: ("Arthritis, Rheumatoid " OR Rheumatoid arthritis OR RA) AND ("Gastrointestinal Microbiome " OR Gastrointestinal Microbiomes OR Microbiome, Gastrointestinal OR Gut Microbiome OR Gut Microbiomes OR Microbiome, Gut). The search strategy for the Embase database is shown in Figure 1. To prevent the omission of the article, two researchers (DWW and XTP) will search the above database independently. Using the snowball method, we manually search for all references contained in the article.

Screening procedures of eligible studies

Once the search is complete, the literature will be managed using EndNote X9 （Clarivate Analytics （US） LLC）. Duplicates will be identified and deleted according to Literature title. Then, the titles and abstracts of the literature will be screened independently by two reviewers (XTP and YFL) according to the inclusion criteria. Retrieval of the full text will be based on the eligible of titles and abstracts, and the literature meeting all the inclusion criteria will be independently assessed. In case of disagreement, a third reviewer (ZLS) will be consulted. To measure interrater agreement, the Kappa coefficients will be both calculated for the processes of titles/

abstract selection and full-text screening. The criteria for judging the scope of the agreement between the evaluators are as follows: 0.00–0.20= slight agreement, 0.21–0.40= fair, 0.41–0.60= moderate, 0.61–0.80= substantial, and 0.81–1.00= almost perfect agreement [60]. The plan of study screening and selection is available in Figure 2.

Assessment of risk of bias

The quality of the included studies will be assessed using NOS [55]. It is a tool mainly used to evaluate the quality of case-control and cohort studies. The parameters considered under each category are ① selection: case definition, representativeness of the cases, selection of controls and definition of controls; ② comparability: comparability of cases and controls based on the basis of the design or analysis; ③ exposure: ascertainment of exposure, the same method of ascertainment for cases and controls, non-response rate. There are 1 to 2 stars in each category, with a maximum of 9 stars for all. The number of stars is proportional to the mass of the study. The number of stars is directly proportional to the quality of the study. The standard of high quality will be NOS score ≥ 7 stars.

To ensure consistency in assessments, the two reviewers (HXG and HZ) will independently evaluate the eligible literature according to NOS and will be summarized in a table. When disagreements arise in the review, the third reviewer (ZLS) cooperates with the team to reach a consensus.

Data extraction

Data from each eligible article will be extracted and compiled using a standardized

excel sheet. Items required for extraction will be obtained the PECOS steps. The following data will be extracted for eligible studies: first author's surname, year of publication, country, classification criteria for RA, number of cases and controls, age and sex, disease duration, antibody positive of RA, 28-joint disease activity score, medication, assessment methods of fecal microbiota, alterations in gut microbial abundance, alpha-diversity indexes (OTUs, Shannon Index and Chao 1 Index) and beta-diversity.

To conduct the meta-analysis, we involve trials that have available and sufficient data to calculate the standardized mean difference (SMD) with 95% confidence interval (CI) in RA patients and healthy controls in the analysis of the pooled data set. If additional data or data transformations will be required for analysis, we will download the publicly available raw data from online repositories or links provided in the original publications. If there is no relevant data in the original literature, we will acquire it after personal communication with the authors of the manuscripts. If the authors do not reply, we will use Web Plot Digitizer (v.4.42) to digitize and extract sufficient data from graphs and plots in the articles [49 61].

To ensure the accuracy of the extracted data, we will randomly select two eligible pieces of literature to be independently extracted by two reviewers (FQC and RZ). Kappa will be applied to compare the consistency of data extraction from the two literatures by the two reviewers. If there is an almost perfect agreement between the two reviewers (Kappa value ≥80%), the remaining literature will extracted by one of the two reviewers.

Data synthesis and analysis

When the number of studies for a single bacterium was five or more, we will conduct the meta-analysis by R language Version 3.4.3 to compare the abundance level of gut microbiota in RA patients with health controls. We will adopt SMD with 95% CI of microbiota abundance as summary statistics when gut microbiota was detected by different techniques in the included studies [62-64]. The included studies will be analyzed at the phylum or genus levels for consitency. The forest plots will be used to visualize the results. We will assess heterogeneity between studies using the Higgin I^2 statistic. In relative terms, I^2 values are proportional to heterogeneity: I^2 values of 25%, 50%, and 75% means low, moderate, and high heterogeneity [65]. Data analysis will be performed by a random-effect model when there is substantial heterogeneity ($I^2 >$ 50%); otherwise, a fixed-effects model will be used [51]. Additionally, we will conduct subgroup analysis of different genders (male/female) and regions (east/west) included in the studies.

If meta-analysis is not feasible, we will conduct narrative synthesis to summarize the relevant evidence between RA and gut dysbiosis. The quantitative narrative synthesis will be conducted according to the Synthesis Without Meta-analysis (SWiM) guideline checklist [66]. In order to define the characteristics of the gut microbiota in RA, we will perform compositional analysis based on the abundance, diversity, and specific bacterial detection of gut microbiota in RA patients and healthy controls.

Assessment of publication bias

We will apply funnel plot and Egger's test to assess publication bias [63]. If funnel plots

present asymmetry, we will use Egger's test to statistically examination [67 68].

Assessment of evidence quality

We will conduct an appraisal of the quality of evidence by applying the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework [69]. Two reviewers (YF and RZ) will assesse five domains including limitations of design, inconsistency, indirectness, imprecision, and publication bias. The GRADE classifies the quality of evidence as 4 levels, high, moderate, low, and very low. Disagreement on the assessment will be resolved by a third reviewer (ZLS).The GRADE Evidence Profiles will be generated using GRADEpro GDT (https://grade pro. org/).

ETHICS AND DISSEMINATION

Ethical approval is unnecessary because the systematic review does not deal with the patient's data and privacy. The findings will be published in a peer-reviewed publication or conference presentations. This systematic review will be a part of the Ph.D. degree research thesis of the primary author (DWW).

PATIENT AND PUBLIC INVOLVEMENT

No patient or public involved.

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Author Statement DWW and XTP drafted the manuscript and contributed equally to this manuscript as joint first authors. YFL provided the materials. HXG and HZ collected and assembled the data. FQC, RZ and YF analyzed and interpreted the data. ZLS conceived the study and critically revised the draft. All authors assisted in manuscript editing and approved its contents.

Funding This work was supported by National Natural Science Foundation of China [81774383], Philosophy and Social Science Research of Jiangsu Higher Education Institutions [2020SJA0335], Post-graduate Research & Practice Innovation Program of Jiangsu Province [KYCX20_1449].

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Word Count: 2,655 words.

Figure1 Embase Session Results

Figure2 Plan of study screening and selection process

Table 1 PRISMA-P 2015 checklist

Section and topic	Item No	Checklist item	Reported on Page #
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	17
Amendments	4	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
Support:			
Sources	5a	Indicate sources of financial or other support for the review	17
Sponsor	5b	Provide name for the review funder and/or sponsor	N/A
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	N/A
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3-6
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
METHODS			

Eligibility criteria	8	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	6-7
Information sources	9	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	8
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	8
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8
Selection process	11b	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)	8
Data collection process	11c	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	9-10
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	10-11
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	11
Risk of bias in individual studies	14	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	9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	10-11
	15b	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 I^2, Kendall's τ)	11
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	11
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	11
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	11
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	11-12

Embase Session Results (22 Oct 2020)

No.	Query	Results
#45	#4 AND #43 AND [english]/lim	817
#44	#4 AND #43	838
#43	#5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR	77807
#42	'bacteria, enteric':ab,ti	20
#41	'enteric bacteria':ab,ti	3639
#40	'flora, intestinal':ab,ti	37
#39	'intestinal flora':ab,ti	5300
#38	'microflora, intestinal':ab,ti	27
#37	'intestine flora':ab,ti	18
#36	'microbiota, intestinal':ab,ti	135
#35	'intestinal microbiotas':ab,ti	28
#34	'intestinal microbiota':ab,ti	9646
#33	'microbiome, intestinal':ab,ti	39
#32	'intestinal microbiomes':ab,ti	75
#31	'intestinal microbiome':ab,ti	2159
#30	'microbiome, gastric':ab,ti	0
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#28	'gastric microbiome':ab,ti	79
#27	'microflora, gastrointestinal':ab,ti	2
#26	'gastrointestinal microflora':ab,ti	306
#25	'microbial community, gastrointestinal':ab,ti	0
#24	'gastrointestinal microbial communities':ab,ti	31
#23	'gastrointestinal microbial community':ab,ti	27
#22	'microbiota, gastrointestinal':ab,ti	14
#21	'gastrointestinal microbiotas':ab,ti	2
#20	'gastrointestinal microbiota':ab,ti	868
#19	'flora, gut':ab,ti	8
#18	'gut flora':ab,ti	2424
#17	'flora, gastrointestinal':ab,ti	6
#16	'gastrointestinal flora':ab,ti	365
#15	'microbiota, gut':ab,ti	605
#14	'gut microbiotas':ab,ti	103
#13	'gut microbiota':ab,ti	24863
#12	'microflora, gut':ab,ti	10
#11	'gut microflora':ab,ti	1766
#10	'microbiome, gut':ab,ti	199
#9	'gut microbiomes':ab,ti	599
#8	'gut microbiome':ab,ti	10019
#7	'microbiome, gastrointestinal':ab,ti	2
#6	'gastrointestinal microbiomes':ab,ti	35
#5	'intestine flora'/exp	60763
#4	#1 OR #2 OR #3	283315
#3	ra:ab,ti	131504
#2	'arthritis, rheumatoid':ab,ti	525
#1	'rheumatoid arthritis'/exp	227296

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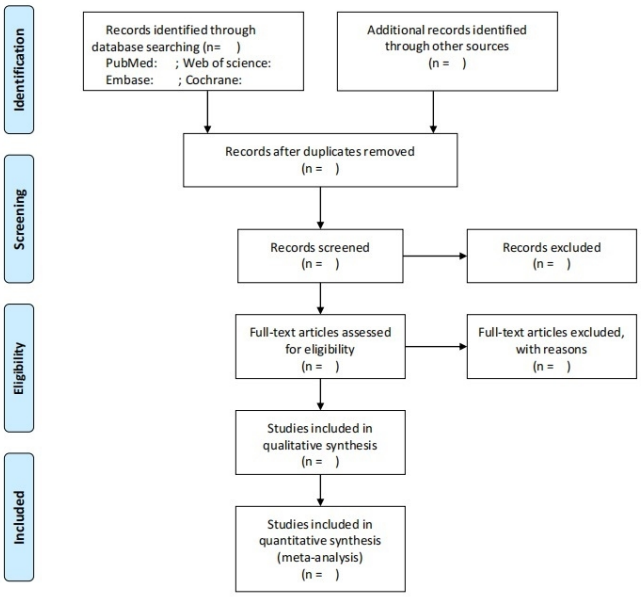

Figure 1 Plan of study screening and selection process

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BMJ Open

Gut Microbial Dysbiosis in Rheumatoid Arthritis: A Systematic Review Protocol of Case-Control Studies

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-052021.R2
Article Type:	Protocol
Date Submitted by the Author:	09-Feb-2022
Complete List of Authors:	Wang, Dan-Wen; Nanjing University of Chinese Medicine, Pang, Xiang-tian; Nanjing University of Chinese Medicine Zhang, Heng; Nanjing University of Chinese Medicine Gao, Hai-xia; Nanjing University of Chinese Medicine Leng, Yu-fei; Shanghai Jiao Tong University School of Medicine, Animal Surgery Laboratory Chen, Feng-qin; Nanjing University of Chinese Medicine Zhang, Rui; Nanjing University of Chinese Medicine Feng, Yun; Nanjing University of Chinese Medicine Sun, Zhi-ling; Nanjing University of Chinese Medicine
Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Rheumatology, Immunology (including allergy)
Keywords:	Rheumatology < INTERNAL MEDICINE, Immunology < NATURAL SCIENCE DISCIPLINES, Microbiology < NATURAL SCIENCE DISCIPLINES

Gut Microbial Dysbiosis in Rheumatoid Arthritis: A Systematic Review Protocol of Case-Control Studies

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Abstract

Introduction: Rheumatoid arthritis (RA) has a huge societal impact due to the high prevalence, irreversible joint damage and systemic complications. Gut microbiota plays an important role in the pathogenesis and progression of RA by regulating the host immune system. Restoring intestinal homeostasis by altering the microbiota could be an attractive strategy for the prevention and treatment of RA. However, the signature features of microbial dysbiosis in RA are still controversial. Therefore, we aim to elucidate the characteristic change in the diversity and composition of gut microbiota in RA.

Methods and analysis: We will systematically search through PubMed, EMBASE, Web of Science and Cochrane Library, as well as dissertations and conference proceedings. The reference lists of all included studies will be also reviewed to retrieve additional relevant studies. The case-control studies that reported either the relative abundance of bacteria at the phylum or genus level or at least one of the alpha-, beta-diversity indexes in both RA and healthy controls will be included. Eligible studies will be screened independently by two reviewers according to the inclusion criteria. The Newcastle-Ottawa Quality Assessment Scale will be used to assess the quality of the included studies. Data extraction, qualitative and quantitative analysis will be performed within the gut microbial dysbiosis in RA. The expected outcomes will be the identification of the specific changes in composition and diversity of the gut microbiota in patients with RA. The quality of evidence will be assessed by the Grading of Recommendations Assessment, Development, and Evaluation framework.

Ethics and dissemination: Ethical approval is unnecessary as this review does not address the data and privacy of patients. The results will be published in a peer-reviewed scientific journal and conference presentations.

PROSPERO registration number: CRD42021225229

Strengths and limitations of this study

This systematic review will identify the characteristic changes in the composition and diversity of gut microbiota in patients with RA, a significant but controversial clinical

issue.

The relative abundances of phyla and/or genus levels in the gut microbiota will be used in this meta-analysis.

The Web Plot Digitizer will be used to digitize and extract data from graphs and plots may lead to biased results.

This systematic review will only include studies written in English, which may limit available data or result in language bias.

INTRODUCTION

RA is a chronic disease characterized by persistent synovitis, inflammatory and autoantibody changes [1]. The prevalence of RA is about 1% globally, and 1.02% in China [2]. The prevalence of RA in women is 2-3 times higher than that in men [3]. Delays in diagnosis and treatment are associated with worse outcomes, including irreversible joint destruction, disability and disease-related non-articular outcomes such as reduced life span [4 5]. In China, 77.6% of RA patients had disabilities, among which moderate and severe disabilities accounted for about 39%, seriously affecting the quality of life of patients [6]. The gradual deterioration of RA leads to a sharp increase in the cost of the disease, which imposes a heavy societal and economic burden on individuals and the country [7-9].

RA is a lifelong condition and currently no cure for most patients [10 11]. European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) recommend that the purpose of RA treatment should be to enable each patient to

achieve the goal of continuous remission or low disease activity [12]. Although the prognosis of RA has improved with advances in diagnosis and treatment in recent decades, the exact etiology and pathogenesis of RA are not fully understood. In order to develop more effective treatment strategies for RA, it is essential to explore its underlying etiology and pathogenesis.

Environmental factors are considered to play an important role in RA [13]. The gut microbiota is considered an important environmental factor in the development of RA [14]. Almost all studies on autoimmune rheumatic diseases show abnormal microbial community structure (i.e. dysbiosis) [15]. Dysbiosis not only affects the pro-inflammatory and anti-inflammatory process of the intestinal mucosa, but also affects the distal joint through the intestinal-joint axis [16-18]. The studies have found dysbiosis in both RA patients and high-risk individuals, indicating that the imbalance of intestinal flora could have occured before the onset of RA [14 19]. Dysbiosis has been involved in the pathogenesis of RA in the decade before its diagnosis [20]. The intestinal flora imbalance also appeared in the initial peak and relapse stage of RA [21]. Dysbiosis is related to the inflammatory response and disease activity of RA, which can be partially recovered by effective treatment [22-24]. As a first-line treatment for RA, methotrexate (MTX) may act in part by modulating the human gut microbiota [24]. The results of animal experiments suggest that interventions targeting intestinal microbiota may have the potential to prevent RA in the preclinical stage [25]. Probiotics supplementation as adjunctive therapy improves the inflammatory state of RA in human and animal studies [26-29]. Therefore, gut microbiota plays an important role in the development of RA, and may be a new

therapeutic target [30 31]. Gut microbiome studies of RA are essential to elucidate etiology and pathophysiological mechanisms and to develop potential therapeutic strategies. Regulating the gut microbiota to slow the progression of the disease, especially in the preclinical phase of RA, may be a promising approach for the treatment of RA in the future [32 33].

Although numerous studies have shown that dysbiosis of the gut microbiome is a key hallmark of RA, the distinct composition of the gut microbiome in RA patients remains controversial. The abundance of *Prevotella* increased in patients with early RA, which hurt the development and prognosis of RA [14 34-37]. However, it has been reported that the abundance of *Prevotella* did not significantly change in RA patients [38]. Moreover, *P. copri* and *P. histicola* of *Prevotella* have different effects on RA [14]. *Bacteroidetes* were enriched in female patients with RA, while *Actinomycetes* and *Collinsella* were enriched in healthy subjects [38]. However, the abundance of *Bacteroides* and *Bifidobacterium* was found to be reduced in RA patients and animal experiments [23 39]. It follows that the results of studies on the gut microbiota of RA patients are contradictory. The identification of specific microbial profiles and patterns that may contribute to the pathogenesis of RA remains a major challenge due to the inconsistent results of studies on the gut microbiota. The conflicting results may stem from inter-study batch effects, such as various biological factors influencing gut microbiome composition, different data processing and analysis methods [40 41]. The differences in demographics of the study cohorts (e.g., sex, age, ethnicity, geography, and diet) also have an important influence on the variability of the results of the gut microbiome study.

Through a quantitative review of the existing literature, the changes of RA gut microbiota can be understood more clearly and comprehensively. Recently, several meta-analyses of gut microbiota have identified specific microbial biomarkers associated with disease [42-47]. However, there has been no systematic review and meta-analysis focusing on the characteristic dysbiosis of gut microbiota in RA to date. Therefore, we will perform a systematic review and meta-analysis to identify characteristic alterations in the gut microbiota of RA patients.

OBJECTIVE

The purpose of this protocol is to outline a systematic review and meta-analysis, which evaluates the changes in the diversity of gut microbiota and the relative abundance of bacterial phyla or genera in patients with RA.

METHODS

Study design

We plan to conduct a systematic review according to the Cochrane Handbook for Systematic Reviews of Interventions Version 6.1 [48], Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA)[49], and PRISMA-Protocols (PRISMA-P) 2015 [50], as well as the Newcastle-Ottawa Quality Assessment Scale (NOS)[51]. The PRISMA-P 2015 checklist is shown in Table 1. This protocol has been registered at PROSPERO (registration number: CRD42021225229).

Eligibility criteria

The studies, written in English as eligible, will be selected and screened based on PECOS steps (Population, Exposure, Comparator, Outcomes, and Study design) [52 53].

The data items will be extracted as following:

Types of participants (P)

The population of interest of the eligible studies should be adults (≥18 years old) with met the diagnostic criteria(the ACR/EULAR 2010) for RA [54] or established RA (1987 classification criteria) [55] in the experimental group， the control group is a healthy population.

Type of exposure (E)

Trials were applied to assess the gut microbiota. Quantitative synthesis of microbiota in fecal samples was performed by using metagenomic shotgun sequencing, 16s rRNA sequencing techniques and/or real-time polymerase chain reaction (rt-PCR).

Comparison (C)

Only healthy adults will be considered eligible for the control group.

Type of outcomes (O)

The primary outcome of the study will be the identification of the composition of the gut microbiome and the relative abundance of bacteria in RA. The secondary outcomes will be considered: changes in the gut microbiota diversity (alpha-diversity, beta-diversity), the effects of different gender and region on the relative abundance of gut microbiota.

Type of studies (S)

We will only include studies with the case-control design, written in English and published in the original peer-reviewed journals. The animal studies, reviews, case

reports, and the full text unachieved will be excluded from the qualitative and quantitative synthesis.

Data sources and search strategies

We conduct the search using the databases Embase, PubMed, Web of Science, and Cochrane Library in the English language published up to September 2020. After reading several documents, a search strategy combining medical subject terms (MeSH) and free words was developed: ("Arthritis, Rheumatoid " OR Rheumatoid arthritis OR RA) AND ("Gastrointestinal Microbiome " OR Gastrointestinal Microbiomes OR Microbiome, Gastrointestinal OR Gut Microbiome OR Gut Microbiomes OR Microbiome, Gut). The search strategy for the Embase database is shown in Figure 1. To prevent the omission of the article, two researchers (DWW and XTP) will search the above database independently. Using the snowball method, we manually search for all references contained in the article.

Screening procedures of eligible studies

Once the search is complete, the literature will be managed using EndNote X9 （Clarivate Analytics （US） LLC）. Duplicates will be identified and deleted according to Literature title. Then, the titles and abstracts of the literature will be screened independently by two reviewers (XTP and YFL) according to the inclusion criteria. Retrieval of the full text will be based on the eligible of titles and abstracts, and the literature meeting all the inclusion criteria will be independently assessed. In case of disagreement, a third reviewer (ZLS) will be consulted. To measure interrater agreement, the Kappa coefficients will be both calculated for the processes of titles/

abstract selection and full-text screening. The criteria for judging the scope of the agreement between the evaluators are as follows: 0.00–0.20= slight agreement, 0.21–0.40= fair, 0.41–0.60= moderate, 0.61–0.80= substantial, and 0.81–1.00= almost perfect agreement [56]. The plan of study screening and selection is available in Figure 2.

Assessment of risk of bias

The quality of the included studies will be assessed using NOS [51]. It is a tool mainly used to evaluate the quality of case-control and cohort studies. The parameters considered under each category are ① selection: case definition, representativeness of the cases, selection of controls and definition of controls; ② comparability: comparability of cases and controls based on the basis of the design or analysis; ③ exposure: ascertainment of exposure, the same method of ascertainment for cases and controls, non-response rate. There are 1 to 2 stars in each category, with a maximum of 9 stars for all. The number of stars is proportional to the mass of the study. The number of stars is directly proportional to the quality of the study. The standard of high quality will be NOS score ≥ 7 stars.

To ensure consistency in assessments, the two reviewers (HXG and HZ) will independently evaluate the eligible literature according to NOS and will be summarized in a table. When disagreements arise in the review, the third reviewer (ZLS) cooperates with the team to reach a consensus.

Data extraction

Data from each eligible article will be extracted and compiled using a standardized excel sheet. Items required for extraction will be obtained the PECOS steps. The

following data will be extracted for eligible studies: first author's surname, year of publication, country, classification criteria for RA, number of cases and controls, age and sex, disease duration, antibody positive of RA, 28-joint disease activity score, medication, assessment methods of fecal microbiota, alterations in gut microbial abundance, alpha-diversity indexes (OTUs, Shannon Index and Chao 1 Index) and beta-diversity.

To conduct the meta-analysis, we involve trials that have available and sufficient data to calculate the standardized mean difference (SMD) with 95% confidence interval (CI) in RA patients and healthy controls in the analysis of the pooled data set. If additional data or data transformations will be required for analysis, we will download the publicly available raw data from online repositories or links provided in the original publications. If there is no relevant data in the original literature, we will acquire it after personal communication with the authors of the manuscripts. If the authors do not reply, we will use Web Plot Digitizer (v.4.42) to digitize and extract sufficient data from graphs and plots in the articles [45 57].

To ensure the accuracy of the extracted data, we will randomly select two eligible pieces of literature to be independently extracted by two reviewers (FQC and RZ). Kappa will be applied to compare the consistency of data extraction from the two literatures by the two reviewers. If there is an almost perfect agreement between the two reviewers (Kappa value ≥80%), the remaining literature will extracted by one of the two reviewers.

Data synthesis and analysis

When the number of studies for a single bacterium is five or more, we will conduct the meta-analysis by R language Version 3.4.3 to compare the abundance level of gut microbiota in RA patients with healthy controls. We will adopt SMD with 95% CI of microbiota abundance as summary statistics when gut microbiota was detected by different techniques in the included studies [58-60]. The included studies will be analyzed at the phylum or genus levels for consitency. The forest plots will be used to visualize the results. We will assess heterogeneity between studies using the Higgin I^2 statistic. In relative terms, I^2 values are proportional to heterogeneity: I^2 values of 25%, 50%, and 75% means low, moderate, and high heterogeneity [61]. Data analysis will be performed by a random-effect model when there is substantial heterogeneity ($I^2 > 50\%$); otherwise, a fixed-effects model will be used [47]. Additionally, we will conduct subgroup analysis of different genders (male/female) and regions (east/west) included in the studies.

If meta-analysis is not feasible, we will conduct narrative synthesis to summarize the relevant evidence between RA and gut dysbiosis. The quantitative narrative synthesis will be conducted according to the Synthesis Without Meta-analysis (SWiM) guideline checklist [62]. In order to define the characteristics of the gut microbiota in RA, we will perform compositional analysis based on the abundance, diversity, and specific bacterial detection of gut microbiota in RA patients and healthy controls.

Assessment of publication bias

We will apply funnel plot and Egger's test to assess publication bias [59]. If funnel plots present asymmetry, we will use Egger's test to statistically examination [63 64].

Assessment of evidence quality

We will conduct an appraisal of the quality of evidence by applying the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework [65]. Two reviewers (YF and RZ) will assesse five domains including limitations of design, inconsistency, indirectness, imprecision, and publication bias. The GRADE classifies the quality of evidence as 4 levels, high, moderate, low, and very low. Disagreement on the assessment will be resolved by a third reviewer (ZLS).The GRADE Evidence Profiles will be generated using GRADEpro GDT (https://grade pro. org/).

ETHICS AND DISSEMINATION

Ethical approval is unnecessary because the systematic review does not deal with the patient's data and privacy. The findings will be published in a peer-reviewed publication or conference presentations. This systematic review will be a part of the Ph.D. degree research thesis of the primary author (DWW).

PATIENT AND PUBLIC INVOLVEMENT

No patient or public involved.

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Author Statement DWW and XTP drafted the manuscript and contributed equally to this manuscript as joint first authors. YFL provided the materials. HXG and HZ collected and assembled the data. FQC, RZ and YF analyzed and interpreted the data. ZLS conceived the study and critically revised the draft. All authors assisted in manuscript editing and approved its contents.

Funding This work was supported by National Natural Science Foundation of China [81774383], Philosophy and Social Science Research of Jiangsu Higher Education Institutions [2020SJA0335], Post-graduate Research & Practice Innovation Program of Jiangsu Province [KYCX20_1449].

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Word Count: 2,655 words.

Figure1 Embase Session Results

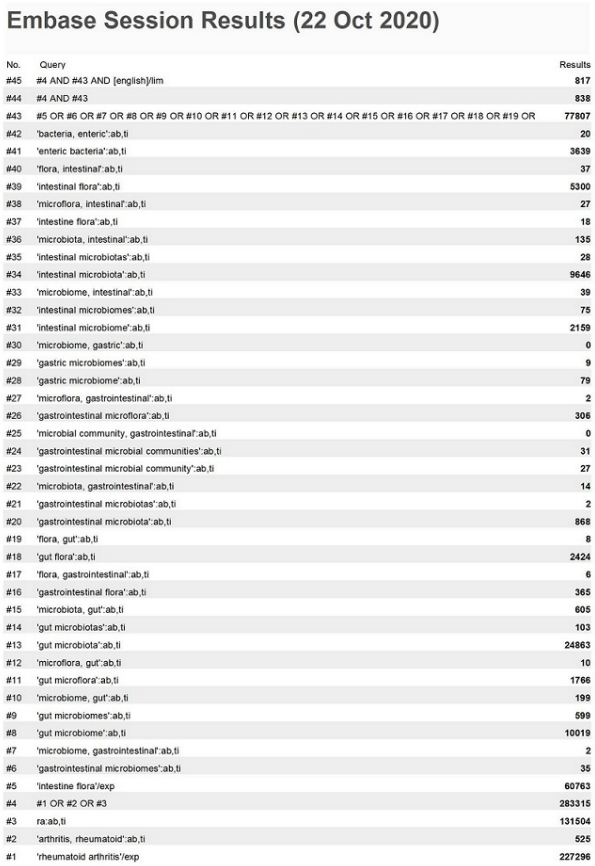
Figure2 Plan of study screening and selection process

Table 1 PRISMA-P 2015 checklist

Section and topic	Item No	Checklist item	Reported on Page #
ADMINISTRATIVE INFORMATION			
Title:			

Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	17
Amendments	4	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
Support:			
Sources	5a	Indicate sources of financial or other support for the review	17
Sponsor	5b	Provide name for the review funder and/or sponsor	N/A
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	N/A
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3-6
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
METHODS			
Eligibility criteria	8	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	6-7
Information sources	9	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	8
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	8
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8
Selection process	11b	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)	8
Data collection process	11c	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	9-10

Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	10-11
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	11
Risk of bias in individual studies	14	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	9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	10-11
	15b	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 I^2, Kendall's τ)	11
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	11
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	11
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	11
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	11-12



Embase Session Results (22 Oct 2020)

No.	Query	Results
#45	#4 AND #43 AND [english]/lim	817
#44	#4 AND #43	838
#43	#5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR	77807
#42	'bacteria, enteric':ab,ti	20
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#40	'flora, intestinal':ab,ti	37
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#23	'gastrointestinal microbial community':ab,ti	27
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#21	'gastrointestinal microbiotas':ab,ti	2
#20	'gastrointestinal microbiota':ab,ti	868
#19	'flora, gut':ab,ti	8
#18	'gut flora':ab,ti	2424
#17	'flora, gastrointestinal':ab,ti	6
#16	'gastrointestinal flora':ab,ti	365
#15	'microbiota, gut':ab,ti	605
#14	'gut microbiotas':ab,ti	103
#13	'gut microbiota':ab,ti	24863
#12	'microflora, gut':ab,ti	10
#11	'gut microflora':ab,ti	1766
#10	'microbiome, gut':ab,ti	199
#9	'gut microbiomes':ab,ti	599
#8	'gut microbiome':ab,ti	10019
#7	'microbiome, gastrointestinal':ab,ti	2
#6	'gastrointestinal microbiomes':ab,ti	35
#5	'intestine flora'/exp	60763
#4	#1 OR #2 OR #3	283315
#3	ra:ab,ti	131504
#2	'arthritis, rheumatoid':ab,ti	525
#1	'rheumatoid arthritis'/exp	227296

Figure1 Embase Session Results

90x90mm (300 x 300 DPI)

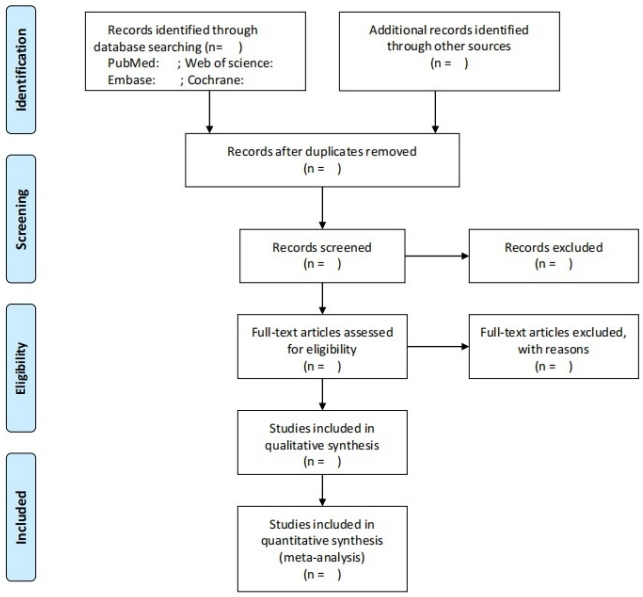

Figure 1 Plan of study screening and selection process

Figure2 Plan of study screening and selection process

90x90mm (300 x 300 DPI)