

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-052021
Article Type:	Protocol
Date Submitted by the Author:	03-Apr-2021
Complete List of Authors:	WANG, DAN-WEN; Nanjing University of Chinese Medicine, Pang, Xiang-tian; Nanjing University of Chinese Medicine Leng, Yu-fei; Nanjing University of Chinese Medicine Gao, Hai-xia; Nanjing University of Chinese Medicine Zhang, Heng; Nanjing University of Chinese Medicine 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
Keywords:	Rheumatology < INTERNAL MEDICINE, Immunology < NATURAL SCIENCE DISCIPLINES, Microbiology < NATURAL SCIENCE DISCIPLINES

SCHOLARONE™
Manuscripts

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

Dan-wen Wang¹, Xiang-tian Pang¹, Yu-fei Leng¹, Hai-xia Gao¹, Heng Zhang¹, Feng-qin Chen¹, Rui Zhang¹, Yun Feng¹, Zhi-ling Sun^{1*}

¹ School of Nursing, Nanjing University of Chinese Medicine, 138 Xianlin Road, Qixia District, Nanjing, Jiangsu Province 210023, China.

* Corresponding author: Zhiling Sun; Email: szl@njucm.edu.cn; telephone number: 13813892093; Address: Nursing College, Nanjing University of Chinese Medicine, 138 Xian Lin Road, Nanjing, Jiangsu Province 210023, China

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

1
2
3
4 (including PubMed, EMBASE, Web of Science and Cochrane Library) have been
5
6 searched and grey literature will also be systematically searched for. Eligible studies
7
8 will be screened independently by two reviewers according to the inclusion
9
10 criteria. The Newcastle-Ottawa Quality Assessment Scale will be used to assess the
11
12 quality of the included studies. Data will be extracted, and meta-analyses will be
13
14 performed within the gut microbial dysbiosis in the rheumatoid arthritis. The quality
15
16 of evidence will be assessed by the Grading of Recommendations Assessment,
17
18 Development, and Evaluation framework.
19
20
21
22
23

24 **Ethics and dissemination:** Ethical approval is unnecessary as this review does not
25
26 address the data and privacy of patients' individuals. The results will be published in a
27
28 peer-reviewed scientific journal and conference presentations.
29
30
31

32 **PROSPERO registration number:** CRD42021225229
33
34
35
36
37

38 **Strengths and limitations of this study**

39
40 This review will elucidate the characteristics of gut dysbiosis in patients with
41
42 rheumatoid arthritis.
43
44

45 The findings of this study will provide a scientific basis for exploring the
46
47 biomolecular link between the gut microbiota and the pathogenesis of rheumatoid
48
49 arthritis.
50
51

52
53 Data pooled may be heterogeneous between studies due to gender, age, diet,
54
55 medication, and specimen measurement methods.
56
57

58 Some studies published in non-English languages may be missed.
59
60

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disease characterized by persistent synovitis, inflammatory and autoantibody changes¹. 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 life⁶. 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 country⁷⁻⁹.

RA is an ancient disease with a complex pathogenesis and currently incurable disease¹⁰. 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 activity¹¹. 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 alleviated¹¹⁻¹⁵. Genetic, environmental and autoimmune factors are considered to play an important role in RA¹⁶. The gut microbiota maintains intestinal mucosal immune function and the integrity of the intestinal mucosal structure and is

1
2
3
4 considered an important environmental factor in the development of RA ¹⁷. Almost all
5
6 studies on autoimmune rheumatic diseases show abnormal microbial community
7
8 structure (i.e. dysbiosis) ¹⁸. Dysbiosis not only affects the pro-inflammatory and
9
10 anti-inflammatory process of intestinal mucosa, but also affects the distal joint
11
12 through the intestinal-joint axis ¹⁹⁻²¹. It is very important to reduce the occurrence of
13
14 RA, delay joint injury and avoid disability, through the improvement of intestinal
15
16 flora imbalance.
17
18

19
20
21
22 The studies have found dysbiosis in RA patients as well as in high-risk individuals,
23
24 indicating that the imbalance of intestinal flora has occurred before the onset of RA ¹⁷
25
26 ²². Dysbiosis has been involved in the pathogenesis of RA in the decade before its
27
28 diagnosis ²³. The intestinal flora imbalance also appeared in the peak and relapse stage
29
30 of RA ²⁴. The dysregulation of intestinal flora is related to the inflammatory response
31
32 and disease activity of RA, which can be partially recovered by effective treatment
33
34 ²⁵⁻²⁷. The results of animal experiments suggest that interventions targeting intestinal
35
36 microbiota may have the potential to prevent RA in the preclinical stage ²⁸. Intestinal
37
38 flora has become a new therapeutic target, which plays an important role in the onset
39
40 and progression of RA ^{29 30} .
41
42

43
44
45
46
47
48 There were significant differences in microbial diversity, species and function of RA
49
50 intestinal flora. The abundance of *Prevotella* increased in patients with early RA,
51
52 which had a negative impact on the development and prognosis of RA ^{17 31-34}.
53
54 However, it has been reported that the abundance of *Prevotella* did not significantly
55
56 change in RA patients ³⁵. Moreover, *P. copri* and *P. histicola* of *Prevotella* have
57
58
59
60

1
2
3
4 different effects on RA ¹⁷. Bacteroidetes were enriched in female patients with RA,
5
6 while Actinomycetes and Collinsella were enriched in healthy subjects ³⁶. However,
7
8 the abundance of Bacteroides and Bifidobacterium was found to be reduced in RA
9
10 patients and animal experiments ^{37 38}. Thus, the results of the study on intestinal flora
11
12 were heterogeneous in RA patients. Through a quantitative review of the existing
13
14 literature, the changes of RA intestinal flora can be understood more clearly and
15
16 comprehensively. However, there have been no systematic reviews and meta-analyses
17
18 on the characteristic changes of intestinal microbiota in RA to date. The purpose of
19
20 this study will be to systematically review the case-control studies on the gut
21
22 microbiota of RA, and use meta-analysis to quantitatively synthesize the results of the
23
24 studies, so as to identify the biomarker of dysbiosis.
25
26
27
28
29
30
31

32 **OBJECTIVE**

33
34 This systematic review attempts to investigate the gut microbiota profiles of RA
35
36 patients by synthesizing available the case-control trains to elucidate the biomarkers
37
38 of dysbiosis with this disorder.
39
40
41
42

43 **METHODS**

44 **Study design**

45
46 We plan to conduct a systematic review according to the Cochrane Handbook for
47
48 Systematic Reviews of Interventions Version 6.1³⁹, Preferred Reporting Items for
49
50 Systematic reviews and Meta-Analyses (PRISMA)⁴⁰, and PRISMA-Protocols
51
52 (PRISMA-P) 2015 ⁴¹, as well as the Newcastle-Ottawa Quality Assessment Scale
53
54 (NOS)⁴². The PRISMA-P 2015 checklist is shown in Table 1. This protocol has been
55
56
57
58
59
60

1
2
3
4 registered at PROSPERO (registration number: CRD42021225229).
5

6 **Eligibility criteria**

7
8
9 The studies, written in English as eligible, will be selected and screened based on
10 PICOS steps (Population, Interventions, Comparator, Outcomes, and Study design).
11

12
13 In this systematic review, PICOS will be scientifically modified by substituting the
14 item "Intervention" for "Investigation". The data items will be extracted as following:
15
16

17 ***Types of participants (P)***

18
19
20 The population of interest of the eligible studies should be adults (≥ 18 years old) with
21 met the diagnostic criteria (the ACR/EULAR 2010) for RA⁴³ or established RA (1987
22 classification criteria)⁴⁴ in the experimental group, the control group is a healthy
23 population.
24
25

26 ***Type of Investigation (I)***

27
28 Trials were applied to assess the gastrointestinal microbiota. Quantitative synthesis of
29 gut microbiota in fecal samples was performed by using metagenomic shotgun
30 sequencing, 16s rRNA sequencing techniques and/or real time polymerase chain
31 reaction (rt-PCR).
32

33 ***Comparison (C)***

34
35 All the following controls will be considered as eligible: healthy population or
36 persons at high risk of RA.
37
38

39 ***Type of outcomes (O)***

40
41 The main results will be taken into account: the composition of intestinal microbiome,
42 changes in the gut microbiota diversity (alpha-diversity, beta-diversity), the relative
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 abundance of opportunistic pathogens and beneficial commensal bacteria. Additional
5
6 outcome measures will be considered: faecal short chain fatty acids (SCFA)
7
8 concentrations, and correlations between clinical, pathological parameters and relative
9
10 abundance of microbial species.
11
12

13 14 ***Type of studies (S)*** 15

16
17 We will only include studies with the design of case-control studies. The original
18
19 peer-reviewed articles written in English are considered. The publication types, such
20
21 as animal studies, reviews, case reports and the full text unachieved will be excluded
22
23 from the qualitative and quantitative synthesis.
24
25

26 27 **Data sources and search strategies** 28

29
30 The search will be conducted using the databases EMBASE, PubMed, Web of Science,
31
32 and Cochrane Library in English language published up to September 2020. After
33
34 reading a number of documents, a search strategy combining medical subject terms
35
36 (MeSH) and free words was developed: ("Arthritis, Rheumatoid " OR Rheumatoid
37
38 arthritis OR RA) AND ("Gastrointestinal Microbiome " OR Gastrointestinal
39
40 Microbiomes OR Microbiome, Gastrointestinal OR Gut Microbiome OR Gut
41
42 Microbiomes OR Microbiome, Gut). In order to prevent the omission of the article,
43
44 two researchers (DWW and XTP) will search the above database independently.
45
46 Using the snowball method, we manually search for all references contained in the
47
48 article.
49
50

51 52 **Screening procedures of eligible studies** 53 54

55
56 Once the search is complete, the literature will be managed using EndNote X9
57
58
59
60

1
2
3
4 (Clarivate Analytics (US) LLC) . Duplicates will be identified and deleted
5
6 according to Literature title. Then, the titles and abstracts of the literature will be
7
8 screened independently by two reviewers (XTP and YFL) according to the inclusion
9
10 criteria. Retrieval of the full text will be based on the eligible of titles and abstracts,
11
12 and the literature meeting all the inclusion criteria will be independently assessed. In
13
14 case of disagreement, a third reviewer (ZLS) will be consulted. To measure interrater
15
16 agreement, the Kappa coefficients will be both calculated for the processes of titles/
17
18 abstract selection and full-text screening. The criteria for judging the scope of the
19
20 agreement between the evaluators are as follows: 0.00–0.20= slight agreement,
21
22 0.21–0.40= fair, 0.41–0.60= moderate, 0.61–0.80=substantial, and 0.81–1.00=almost
23
24 perfect agreement⁴⁵. The plan of study screening and selection is available in Figure
25
26
27
28
29
30
31
32
33 1.

34 35 **Assessment of risk of bias**

36
37 The quality of the included studies will be assessed using NOS ⁴². It is a tool mainly
38
39 used to evaluate the quality of case-control and cohort studies. The parameters
40
41 considered under each category are: ① selection: case definition, representativeness
42
43 of the cases, selection of controls and definition of controls; ② comparability:
44
45 comparability of cases and controls on the basis of the design or analysis; ③
46
47 exposure: ascertainment of exposure, same method of ascertainment for cases and
48
49 controls, non-response rate. There are 1 to 2 stars in each category, with a maximum
50
51 of 9 stars for all. The number of stars is proportional to the mass of the study. The
52
53 number of stars is directly proportional to the quality of the study. The standard of
54
55
56
57
58
59
60

1
2
3
4 high quality will be NOS score ≥ 7 stars.
5

6 To ensure consistency in assessments, the two reviewers (HXG and HZ) will
7 independently evaluate the eligible literature according to NOS and will be
8 summarized in a table. If disagreements arise in the review, they will be resolved by
9 the third reviewer (ZLS) in collaboration with the team to reach consensus.
10
11
12
13
14
15

16 **Data extraction**

17 Data from each eligible article will be extracted and compiled using a standardized
18 excel sheet. Items required for extraction will be obtained the PICOS steps. The
19 following data will be extracted for eligible studies: first author's surname, year of
20 publication, country, classification criteria for RA, number of cases and controls, age
21 and sex, disease extent, antibody positive of RA, 28-joint disease activity score,
22 medication, assessment methods of fecal microbiota, SCFA concentrations,
23 alterations in gut microbial diversity. To ensure the accuracy of the extracted data, we
24 will randomly select two eligible literatures, which will be independently extracted by
25 two reviewers (FQC and RZ). Kappa will be applied to compare the consistency of
26 data extraction from the two literatures by the two reviewers. If there is an almost
27 perfect agreement between the two reviewers (Kappa value $\geq 80\%$), the remaining
28 literature will extracted by one of the two reviewers.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

50 **Data synthesis and analysis**

51 The included literature will reported the percentage of gut bacteria, also known as
52 relative abundance, in RA patients and controls. If sufficient data will be available to
53 calculate a pooled effect estimate in eligible studies, we will consider conducting a
54
55
56
57
58
59
60

1
2
3
4 meta-analysis. We will standardize all extracted data. In turn the relative abundance
5
6 and standard error from each study will be used to obtain the total percentage of
7
8 bacteria of different phyla and genera in RA patients and controls. To clarify the
9
10 diversity changes in bacteria between RA and healthy people, we will calculate their
11
12 percentages for the phyla and genera of each differential bacterium between the two
13
14 groups. A random effects meta-analysis will be performed using Review Manager 5.3
15
16 software (the Cochrane Collaboration, Copenhagen, Denmark)⁴⁶. We will use forest
17
18 plots to visualize the results. We will assess heterogeneity between studies using the
19
20 Higgins I² statistic. In relative terms, I² values are proportional to heterogeneity: I²
21
22 values of 0-30% means minimal heterogeneity, 31-50% means moderate
23
24 heterogeneity, and > 50% means substantial heterogeneity⁴⁷. If meta-analysis is not
25
26 feasible, we will conduct narrative synthesis to summarize the relevant evidence
27
28 between RA and gut dysbiosis.
29
30
31
32
33
34
35
36

37 **Assessment of publication bias**

38
39
40 We will apply Begg's funnel plot and Egger's test to assess publication bias⁴⁸.
41
42
43 Publication bias will be considered if there is an asymmetrically shaped Begg's funnel
44
45 plot or Egger's test p-value < 0.10.
46
47

48 **Assessment of evidence quality**

49
50
51 We will conduct an assessment of the quality of evidence by applying the Grading of
52
53 Recommendations Assessment, Development, and Evaluation (GRADE) framework
54
55
56⁴⁷. Five domains will be assessed by two reviewers (YF and RZ), which is limitations
57
58 of design, inconsistency, indirectness, imprecision and publication bias. The GRADE
59
60

1
2
3
4 classifies the quality of evidence into 4 levels, high, moderate, low, and very low.
5

6 Disagreement on the assessment will be resolved by a third reviewer (ZLS).The
7

8
9 GRADE Evidence Profiles will be generated using GRADEpro GDT (<https://grade>
10
11
12 [pro. org/](https://grade)).
13

14 **ETHICS AND DISSEMINATION**

15
16 Ethical approval is not necessary because the systematic review does not deal with the
17
18 patient's personal data and privacy. The findings will be published in a peer-reviewed
19
20 publication and conference presentations. This systematic review will be included as a
21
22 chapter in the primary author's (DWW) PhD degree research thesis.
23
24
25

26 **PATIENT AND PUBLIC INVOLVEMENT**

27
28 No patient or public involved.
29
30
31
32
33
34

35 **References**

- 36
37 1. Aletaha D, Smolen JS. Diagnosis and Management of Rheumatoid Arthritis: A Review. *JAMA*
38 2018;320(13):1360-72. doi: 10.1001/jama.2018.13103 [published Online First: 2018/10/05]
39
- 40 2. Gerlag DM, Norris JM, Tak PP. Towards prevention of autoantibody-positive rheumatoid arthritis:
41 from lifestyle modification to preventive treatment. *Rheumatology (Oxford)*
42 2016;55(4):607-14. doi: 10.1093/rheumatology/kev347 [published Online First: 2015/09/17]
43
- 44 3. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. *Autoimmun Rev*
45 2005;4(3):130-6. doi: 10.1016/j.autrev.2004.09.002 [published Online First: 2005/04/13]
46
- 47 4. Taskforce ELAR. Rheuma Map A Research Roadmap to transform the lives of people with
48 Rheumatic and Musculoskeletal Diseases. 2019
- 49 5. Veale DJ, Orr C, Fearon U. Cellular and molecular perspectives in rheumatoid arthritis. *Semin*
50 *Immunopathol* 2017;39(4):343-54. doi: 10.1007/s00281-017-0633-1 [published Online First:
51 2017/05/17]
52
- 53 6. Zhou Y, Wang X, An Y, et al. Disability and health-related quality of life in Chinese patients with
54 rheumatoid arthritis: A cross-sectional study. *Int J Rheum Dis* 2018;21(9):1709-15. doi:
55 10.1111/1756-185X.13345 [published Online First: 2018/10/23]
56
- 57 7. Hu H, Luan L, Yang K, et al. Burden of rheumatoid arthritis from a societal perspective: A
58 prevalence-based study on cost of this illness for patients in China. *Int J Rheum Dis*
59 2018;21(8):1572-80. doi: 10.1111/1756-185X.13028 [published Online First: 2017/02/18]
60

- 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
 - 16
 - 17
 - 18
 - 19
 - 20
 - 21
 - 22
 - 23
 - 24
 - 25
 - 26
 - 27
 - 28
 - 29
 - 30
 - 31
 - 32
 - 33
 - 34
 - 35
 - 36
 - 37
 - 38
 - 39
 - 40
 - 41
 - 42
 - 43
 - 44
 - 45
 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60
8. Furneri G, Mantovani LG, Belisari A, et al. Systematic literature review on economic implications and pharmaco-economic issues of rheumatoid arthritis. *Clinical and experimental rheumatology* 2012;30(4 Suppl 73):S72-84. [published Online First: 2012/10/18]
9. Shafrin J, Tebeka MG, Price K, et al. The Economic Burden of ACPA-Positive Status Among Patients with Rheumatoid Arthritis. *J Manag Care Spec Pharm* 2018;24(1):4-11. doi: 10.18553/jmcp.2017.17129 [published Online First: 2018/01/02]
10. Alam J, Jantan I, Bukhari SNA. Rheumatoid arthritis: Recent advances on its etiology, role of cytokines and pharmacotherapy. *Biomed Pharmacother* 2017;92:615-33. doi: 10.1016/j.biopha.2017.05.055 [published Online First: 2017/06/06]
11. Smolen JS, Landewe RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. *Ann Rheum Dis* 2020 doi: 10.1136/annrheumdis-2019-216655 [published Online First: 2020/01/24]
12. Jakubovic BD, Donovan A, Webster PM, et al. Methotrexate-induced pulmonary toxicity. *Can Respir J* 2013;20(3):153-5. doi: 10.1155/2013/527912 [published Online First: 2013/06/14]
13. Huang RY, Pan HD, Wu JQ, et al. Comparison of combination therapy with methotrexate and sinomenine or leflunomide for active rheumatoid arthritis: A randomized controlled clinical trial. *Phytomedicine* 2019;57:403-10. doi: 10.1016/j.phymed.2018.12.030 [published Online First: 2019/03/10]
14. Lee EB, Fleischmann R, Hall S, et al. Tofacitinib versus methotrexate in rheumatoid arthritis. *N Engl J Med* 2014;370(25):2377-86. doi: 10.1056/NEJMoa1310476 [published Online First: 2014/06/19]
15. Ramiro S, Sepriano A, Chatzidionysiou K, et al. Safety of synthetic and biological DMARDs: a systematic literature review informing the 2016 update of the EULAR recommendations for management of rheumatoid arthritis. *Ann Rheum Dis* 2017;76(6):1101-36. doi: 10.1136/annrheumdis-2016-210708 [published Online First: 2017/03/17]
16. Karami J, Aslani S, Jamshidi A, et al. Genetic implications in the pathogenesis of rheumatoid arthritis; an updated review. *Gene* 2019;702:8-16. doi: 10.1016/j.gene.2019.03.033 [published Online First: 2019/03/25]
17. Maeda Y, Takeda K. Host-microbiota interactions in rheumatoid arthritis. *Exp Mol Med* 2019;51(12):1-6. doi: 10.1038/s12276-019-0283-6 [published Online First: 2019/12/13]
18. Konig MF. The microbiome in autoimmune rheumatic disease. *Best Pract Res Clin Rheumatol* 2020:101473. doi: 10.1016/j.berh.2019.101473 [published Online First: 2020/02/12]
19. Hager J, Bang H, Hagen M, et al. The Role of Dietary Fiber in Rheumatoid Arthritis Patients: A Feasibility Study. *Nutrients* 2019;11(10) doi: 10.3390/nu11102392 [published Online First: 2019/10/09]
20. Van de Wiele T, Van Praet JT, Marzorati M, et al. How the microbiota shapes rheumatic diseases. *Nat Rev Rheumatol* 2016;12(7):398-411. doi: 10.1038/nrrheum.2016.85 [published Online First: 2016/06/17]
21. Sun Y, Chen Q, Lin P, et al. Characteristics of Gut Microbiota in Patients With Rheumatoid Arthritis in Shanghai, China. *Frontiers in Cellular and Infection Microbiology* 2019;9:369. doi: 10.3389/fcimb.2019.00369 [published Online First: 2019/11/12]
22. Alpizar-Rodriguez D, Lesker TR, Gronow A, et al. *Prevotella copri* in individuals at risk for rheumatoid arthritis. *Ann Rheum Dis* 2019;78(5):590-93. doi:

- 1
2
3 10.1136/annrheumdis-2018-214514 [published Online First: 2019/02/15]
4
5 23. Nguyen Y, Mariette X, Salliot C, et al. Chronic diarrhoea and risk of rheumatoid arthritis: findings
6 from the French E3N-EPIC Cohort Study. *Rheumatology (Oxford)* 2020 doi:
7 10.1093/rheumatology/keaa133 [published Online First: 2020/05/18]
8
9 24. Nemoto N, Takeda Y, Nara H, et al. Analysis of intestinal immunity and flora in a collagen-induced
10 mouse arthritis model: differences during arthritis progression. *Int Immunol*
11 2020;32(1):49-56. doi: 10.1093/intimm/dxz058 [published Online First: 2019/09/29]
12
13 25. Zhang X, Zhang D, Jia H, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis
14 and partly normalized after treatment. *Nat Med* 2015;21(8):895-905. doi: 10.1038/nm.3914
15 [published Online First: 2015/07/28]
16
17 26. Chiang HI, Li JR, Liu CC, et al. An Association of Gut Microbiota with Different Phenotypes in
18 Chinese Patients with Rheumatoid Arthritis. *J Clin Med* 2019;8(11) doi: 10.3390/jcm8111770
19 [published Online First: 2019/10/28]
20
21 27. Nayak RR, Alexander M, Stapleton-Grey K, et al. Perturbation of the human gut microbiome by a
22 non-antibiotic drug contributes to the resolution of autoimmune disease. *bioRxiv* 2019 doi:
23 10.1101/600155
24
25 28. Jubair WK, Hendrickson JD, Severs EL, et al. Modulation of Inflammatory Arthritis in Mice by Gut
26 Microbiota Through Mucosal Inflammation and Autoantibody Generation. *Arthritis*
27 *Rheumatol* 2018;70(8):1220-33. doi: 10.1002/art.40490 [published Online First: 2018/03/14]
28
29 29. Lee YH. Causal association of gut microbiome on the risk of rheumatoid arthritis: a Mendelian
30 randomisation study. *Ann Rheum Dis* 2020 doi: 10.1136/annrheumdis-2019-216747
31 [published Online First: 2020/01/12]
32
33 30. Uchiyama K, Naito Y, Takagi T. Intestinal microbiome as a novel therapeutic target for local and
34 systemic inflammation. *Pharmacology & therapeutics* 2019;199:164-72. doi:
35 10.1016/j.pharmthera.2019.03.006 [published Online First: 2019/03/17]
36
37 31. Drago L. Prevotella Copri and Microbiota in Rheumatoid Arthritis: Fully Convincing Evidence? *J Clin*
38 *Med* 2019;8(11) doi: 10.3390/jcm8111837 [published Online First: 2019/11/07]
39
40 32. Pianta A, Arvikar S, Strle K, et al. Evidence of the Immune Relevance of Prevotella copri, a Gut
41 Microbe, in Patients With Rheumatoid Arthritis. *Arthritis Rheumatol* 2017;69(5):964-75. doi:
42 10.1002/art.40003 [published Online First: 2016/11/20]
43
44 33. Lorenzo D, GianVincenzo Z, Carlo Luca R, et al. Oral-Gut Microbiota and Arthritis: Is There an
45 Evidence-Based Axis? *J Clin Med* 2019;8(10) doi: 10.3390/jcm8101753 [published Online First:
46 2019/10/28]
47
48 34. Maeda Y, Takeda K. Role of Gut Microbiota in Rheumatoid Arthritis. *J Clin Med* 2017;6(6) doi:
49 10.3390/jcm6060060 [published Online First: 2017/06/10]
50
51 35. Jeong Y, Kim JW, You HJ, et al. Gut Microbial Composition and Function Are Altered in Patients
52 with Early Rheumatoid Arthritis. *J Clin Med* 2019;8(5) doi: 10.3390/jcm8050693 [published
53 Online First: 2019/05/19]
54
55 36. Y J, JW K, HJ Y, et al. Gut Microbial Composition and Function Are Altered in Patients with Early
56 Rheumatoid Arthritis. 2019;8(5) doi: 10.3390/jcm8050693
57
58 37. Li X, Lu C, Fan D, et al. Human Umbilical Mesenchymal Stem Cells Display Therapeutic Potential in
59 Rheumatoid Arthritis by Regulating Interactions Between Immunity and Gut Microbiota via
60 the Aryl Hydrocarbon Receptor. *Frontiers in cell and developmental biology* 2020;8 doi:
10.3389/fcell.2020.00131

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
38. Chiang H-I, Li J-R, Liu C-C, et al. An Association of Gut Microbiota with Different Phenotypes in Chinese Patients with Rheumatoid Arthritis. *Journal of Clinical Medicine* 2019;8(11) doi: 10.3390/jcm8111770
 39. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA. Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020). www.training.cochrane.org/handbook. www.training.cochrane.org/handbook.
 40. Moher D, Liberati, A., Tetzlaff, J., & Altman, D. G.; PRISMA Group.(2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Annals of Internal Medicine* 2009;151(4):264-69. doi: 10.7326/0003-4819-151-4-200908180-00135 %m 19622511
 41. Shamseer L, Moher D, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. 2015;349:g7647. doi: 10.1136/bmj.g7647 %J BMJ : British Medical Journal
 42. GA Wells BS, D O'Connell, J Peterson, V Welch, M Losos, P Tugwell,. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
 43. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010;62(9):2569-81. doi: 10.1002/art.27584 [published Online First: 2010/09/28]
 44. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31(3):315-24. doi: 10.1002/art.1780310302 [published Online First: 1988/03/01]
 45. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33(1):159-74. [published Online First: 1977/03/01]
 46. Iglesias-Vazquez L, Van Ginkel Riba G, Arija V, et al. Composition of Gut Microbiota in Children with Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Nutrients* 2020;12(3) doi: 10.3390/nu12030792 [published Online First: 2020/03/21]
 47. Bobbio E, Lingbrant M, Nwaru BI, et al. Inflammatory cardiomyopathies: short- and long-term outcomes after heart transplantation-a protocol for a systematic review and meta-analysis. *Heart Fail Rev* 2020;25(3):481-85. doi: 10.1007/s10741-020-09919-x [published Online First: 2020/01/15]
 48. Kim KN, Yao Y, Ju SY. Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans with Obesity: A Systematic Review and Meta-Analysis. *Nutrients* 2019;11(10) doi: 10.3390/nu11102512 [published Online First: 2019/10/23]

52 **Author Statement** DWW and XTP drafted the manuscript and contributed equally to
53
54 this manuscript as joint first authors. YFL provided the materials. HXG and HZ
55
56 collected and assembled the data. FQC, RZ and YF analyzed and interpreted the
57
58 data. ZLS conceived the study and critically revised the draft. All authors assisted in
59
60

1
2
3
4 manuscript editing and approved its contents.
5

6 **Funding** This work was supported by National Natural Science Foundation
7 of China [81774383], Philosophy and Social Science Research of Jiangsu Higher
8 Education Institutions [2020SJA0335], Post-graduate Research & Practice Innovation
9 Program of Jiangsu Province [KYCX20_1449, KYCX20_1616], Qinglan Project
10 Foundation of Jiangsu Province, Nursing Professional Innovation Practice and
11 Teaching Team Open Fund of Nanjing University of Chinese Medicine
12 [NZYHLXPPQL2019-26].
13
14

15 **Competing interests** None declared.
16

17 **Patient consent for publication** Not required.
18

19 **Provenance and peer review** Not commissioned; externally peer reviewed.
20
21

22 **Word Count:** 1,989 words.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

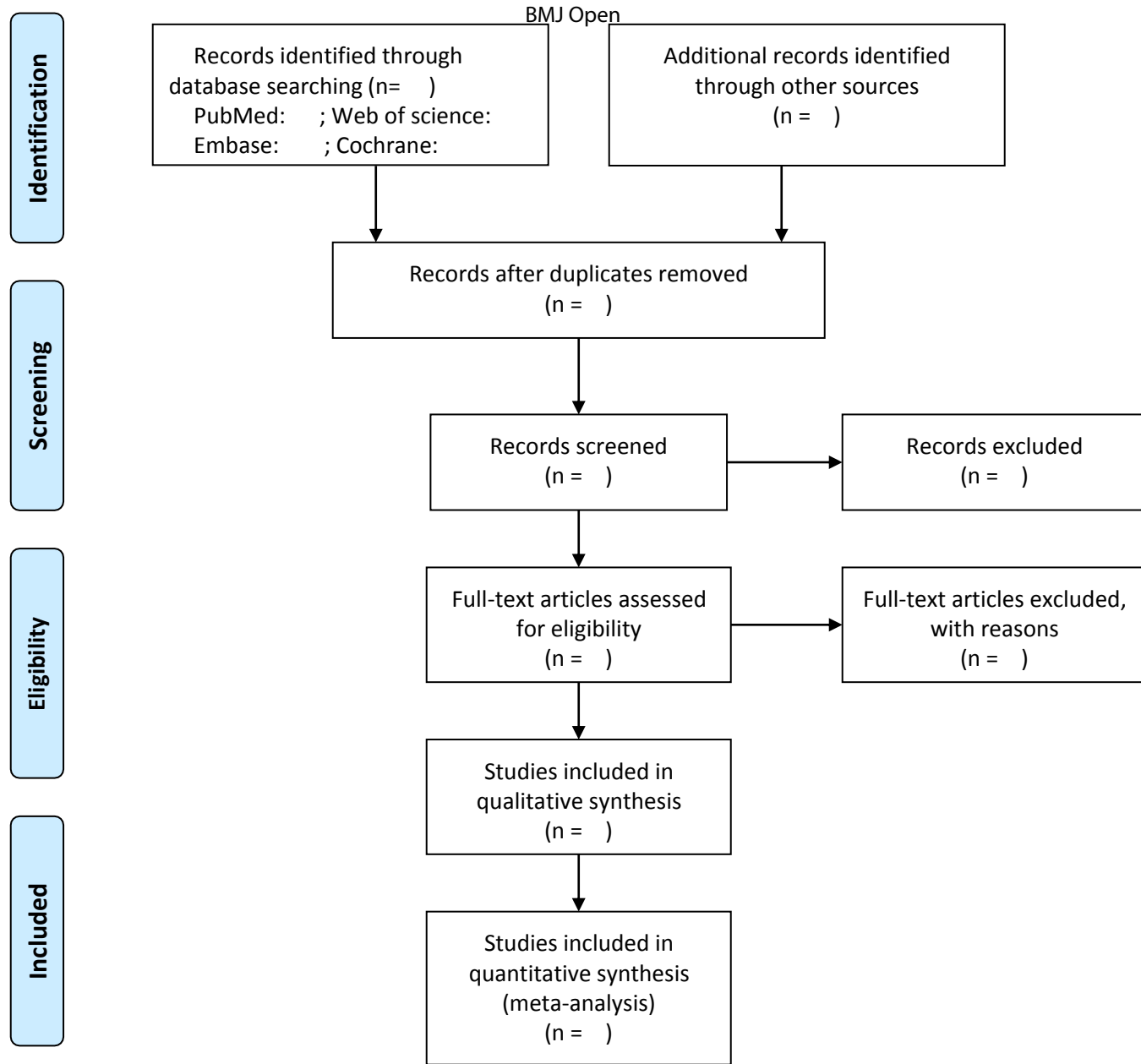


Table 1 PRISMA-P 2015 checklist

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:	<i>BMJ Open</i>
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

SCHOLARONE™
Manuscripts

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

Dan-wen Wang ^{1#}, Xiang-tian Pang ^{1#}, Heng Zhang ¹, Hai-xia Gao ¹, Yu-fei Leng ², Feng-qin Chen ¹, Rui Zhang ¹, Yun Feng ¹, Zhi-ling Sun ^{1*}

1 School of Nursing, Nanjing University of Chinese Medicine, 138 Xianlin Road, Qixia District, Nanjing, Jiangsu Province 210023, China.

2 Animal Surgery Laboratory, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China

Dan-wen Wang and Xiang-tian Pang are joint first authors.

* Corresponding author: Zhiling Sun; Email: szl@njucm.edu.cn; telephone number: 13813892093; Address: Nursing College, Nanjing University of Chinese Medicine, 138 Xian Lin Road, Nanjing, Jiangsu Province 210023, China

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.

1
2
3
4 **Methods and analysis:** We will systematically search through PubMed, EMBASE,
5
6 Web of Science and Cochrane Library, as well as dissertations and conference
7
8 proceedings. The reference lists of all included studies will be also reviewed
9
10 to retrieve additional relevant studies. The case-control studies that reported
11
12 either the relative abundance of bacteria at the phylum or genus level or at
13
14 least one of the alpha-, beta-diversity indexes in both RA and health controls
15
16 will be included. Eligible studies will be screened independently by two reviewers
17
18 according to the inclusion criteria. The Newcastle-Ottawa Quality Assessment Scale
19
20 will be used to assess the quality of the included studies. Data extraction, qualitative
21
22 and quantitative analysis will be performed within the gut microbial dysbiosis in RA.
23
24 The expected outcomes will be the specific changes in composition and
25
26 diversity of the gut microbiota in patients with RA. The quality of evidence will
27
28 be assessed by the Grading of Recommendations Assessment, Development, and
29
30 Evaluation framework.
31
32
33
34
35
36
37
38

39
40 **Ethics and dissemination:** Ethical approval is unnecessary as this review does not
41
42 address the data and privacy of patients. The results will be published in a
43
44 peer-reviewed scientific journal and conference presentations.
45
46
47

48 **PROSPERO registration number:** CRD42021225229
49
50
51

52 53 **Strengths and limitations of this study**

54
55 This systematic review will identify the characteristic changes in the composition and
56
57 diversity of gut microbiota in patients with RA, a significant but controversial clinical
58
59
60

1
2
3
4 issue.

5
6 The percentage and relative abundance of phyla or genus levels in the gut microbiota
7
8
9 will be used in this analysis to avoid potential variation due to different detection
10
11
12 methods of the microbiome in the included studies.

13
14 The Web Plot Digitizer will be used to digitize and extract data from graphs and plots
15
16
17 may lead to biased results.

18
19 This systematic review will only include studies written in English, which may limit
20
21
22 available data or result in language bias.
23
24
25

26 27 **INTRODUCTION**

28
29
30 RA is a chronic disease characterized by persistent synovitis, inflammatory and
31
32 autoantibody changes ¹. The prevalence of RA is about 1% globally, and 1.02% in
33
34 China ². The prevalence of RA in women is 2-3 times higher than that in men ³.
35
36 Delays in diagnosis and treatment are associated with worse outcomes,
37
38 including irreversible joint destruction, disability and disease-related non-articular
39
40 outcomes such as reduced life span ^{4,5}. In China, 77.6% of RA patients had disabilities,
41
42 among which moderate and severe disabilities accounted for about 39%, seriously
43
44 affecting the quality of life of patients ⁶. The gradual deterioration of RA leads to a
45
46 sharp increase in the cost of the disease, which imposes a heavy societal and
47
48 economic burden on individuals and the country ⁷⁻⁹.
49
50
51
52
53
54
55

56
57 RA is an ancient disease with a complex pathogenesis and is currently an incurable
58
59 disease ¹⁰. European League Against Rheumatism (EULAR) and American College of
60

1
2
3
4 Rheumatology (ACR) recommend that the purpose of RA treatment should be to
5
6 enable each patient to achieve the goal of continuous remission or low disease activity
7
8
9 ¹¹. The prognosis of RA has improved in recent decades with advances in diagnosis
10
11 and treatment. However, as the etiology and pathogenesis of RA are not fully
12
13 understood, the therapeutic effect is greatly reduced, which seriously hinders the
14
15 effective remission of RA patients ¹¹⁻¹⁵. Therefore, it is particularly important to
16
17 explore the etiology and pathogenesis of RA.
18
19
20

21
22
23 Environmental factors are considered to play an important role in RA ¹⁶. The gut
24
25 microbiota is considered an important environmental factor in the development of RA
26
27 ¹⁷. Almost all studies on autoimmune rheumatic diseases show abnormal microbial
28
29 community structure (i.e. dysbiosis) ¹⁸. Dysbiosis not only affects the
30
31 pro-inflammatory and anti-inflammatory process of the intestinal mucosa, but also
32
33 affects the distal joint through the intestinal-joint axis ¹⁹⁻²¹. The studies have found
34
35 dysbiosis in both RA patients and high-risk individuals, indicating that the imbalance
36
37 of intestinal flora has occurred before the onset of RA ^{17 22}. Dysbiosis has been
38
39 involved in the pathogenesis of RA in the decade before its diagnosis ²³. The intestinal
40
41 flora imbalance also appeared in the initial peak and relapse stage of RA ²⁴. Dysbiosis
42
43 is related to the inflammatory response and disease activity of RA, which can be
44
45 partially recovered by effective treatment ²⁵⁻²⁷. As a first-line treatment for RA,
46
47 methotrexate (MTX) may act in part by modulating the human gut microbiota ²⁷. The
48
49 results of animal experiments suggest that interventions targeting intestinal microbiota
50
51 may have the potential to prevent RA in the preclinical stage ²⁸. Probiotics
52
53
54
55
56
57
58
59
60

1
2
3
4 supplementation as adjunctive therapy improves the inflammatory state of RA in
5
6 human and animal studies ²⁹⁻³². Therefore, gut microbiota plays an important role in
7
8 the development of RA, and may be a new therapeutic target ^{33 34}. Gut microbiome
9
10 studies of RA are essential to elucidate etiology and pathophysiological mechanisms
11
12 and to develop potential therapeutic strategies. Regulating the gut microbiota to slow
13
14 the progression of the disease, especially in the preclinical phase of RA, may be a
15
16 promising approach for the treatment of RA in the future ^{35 36}.

17
18
19
20
21
22 Although numerous studies have shown that dysbiosis of the gut microbiome is a key
23
24 hallmark of RA, the distinct composition of the gut microbiome in RA patients
25
26 remains controversial. The abundance of *Prevotella* increased in patients with early
27
28 RA, which hurt the development and prognosis of RA ^{17 37-40}. However, it has been
29
30 reported that the abundance of *Prevotella* did not significantly change in RA patients
31
32 ⁴¹. Moreover, *P. copri* and *P. histicola* of *Prevotella* have different effects on RA ¹⁷.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
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}.

1
2
3
4 Through a quantitative review of the existing literature, the changes of RA gut
5
6 microbiota can be understood more clearly and comprehensively. Recently, several
7
8 meta-analyses of gut microbiota have identified specific microbial biomarkers
9
10 associated with disease ⁴⁶⁻⁵¹. However, there has been no systematic review and
11
12 meta-analysis focusing on the characteristic dysbiosis of gut microbiota in RA to date.
13
14
15 Therefore, we will perform a systematic review and meta-analysis to identify
16
17 characteristic alterations in the gut microbiota of RA patients.
18
19
20
21

22 **OBJECTIVE**

23
24 The purpose of this protocol is to outline a systematic review and meta-analysis,
25
26 which evaluates the changes in the diversity of gut microbiota and the relative
27
28 abundance of bacterial phyla or genera in patients with RA.
29
30
31

32 **METHODS**

33 **Study design**

34
35 We plan to conduct a systematic review according to the Cochrane Handbook for
36
37 Systematic Reviews of Interventions Version 6.1 ⁵², Preferred Reporting Items for
38
39 Systematic reviews and Meta-Analyses (PRISMA)⁵³, and PRISMA-Protocols
40
41 (PRISMA-P) 2015 ⁵⁴, as well as the Newcastle-Ottawa Quality Assessment Scale
42
43 (NOS)⁵⁵. The PRISMA-P 2015 checklist is shown in Table 1. This protocol has been
44
45 registered at PROSPERO (registration number: CRD42021225229).
46
47
48
49
50
51

52 **Eligibility criteria**

53
54 The studies, written in English as eligible, will be selected and screened based on
55
56 PECOS steps (Population, Exposure, Comparator, Outcomes, and Study design) ^{56 57}.
57
58
59
60

1
2
3
4 The data items will be extracted as following:
5

6
7 ***Types of participants (P)***
8

9 The population of interest of the eligible studies should be adults (≥ 18 years old) with
10 met the diagnostic criteria (the ACR/EULAR 2010) for RA⁵⁸ or established RA (1987
11 classification criteria)⁵⁹ in the experimental group, the control group is a healthy
12 population.
13
14
15
16
17
18
19

20
21 ***Type of exposure (E)***
22

23 Trials were applied to assess the gut microbiota. Quantitative synthesis of microbiota
24 in fecal samples was performed by using metagenomic shotgun sequencing, 16s
25 rRNA sequencing techniques and/or real-time polymerase chain reaction (rt-PCR).
26
27
28
29

30
31 ***Comparison (C)***
32

33 Only healthy adults will be considered eligible for the control group.
34
35

36
37 ***Type of outcomes (O)***
38

39 The primary outcome of the study will be the composition of the gut microbiome and
40 the relative abundance of bacteria in RA. The secondary outcomes will be considered:
41 changes in the gut microbiota diversity (alpha-diversity, beta-diversity), the effects of
42 different gender and region on the relative abundance of gut microbiota.
43
44
45
46
47
48

49
50 ***Type of studies (S)***
51

52 We will only include studies with the case-control design, written in English and
53 published in the original peer-reviewed journals. The animal studies, reviews, case
54
55
56
57
58
59
60

1
2
3
4 reports, and the full text unachieved will be excluded from the qualitative and
5
6 quantitative synthesis.
7

8 9 **Data sources and search strategies**

10
11 We conduct the search using the databases Embase, PubMed, Web of Science, and
12
13 Cochrane Library in the English language published up to September 2020. After
14
15 reading several documents, a search strategy combining medical subject terms (MeSH)
16
17 and free words was developed: ("Arthritis, Rheumatoid " OR Rheumatoid arthritis OR
18
19 RA) AND ("Gastrointestinal Microbiome " OR Gastrointestinal Microbiomes OR
20
21 Microbiome, Gastrointestinal OR Gut Microbiome OR Gut Microbiomes OR
22
23 Microbiome, Gut). The search strategy for the Embase database is shown in Figure 1.
24
25 To prevent the omission of the article, two researchers (DWW and XTP) will search
26
27 the above database independently. Using the snowball method, we manually search
28
29 for all references contained in the article.
30
31
32
33
34
35
36

37 38 **Screening procedures of eligible studies**

39
40 Once the search is complete, the literature will be managed using EndNote X9
41
42 (Clarivate Analytics (US) LLC) . Duplicates will be identified and deleted
43
44 according to Literature title. Then, the titles and abstracts of the literature will be
45
46 screened independently by two reviewers (XTP and YFL) according to the inclusion
47
48 criteria. Retrieval of the full text will be based on the eligible of titles and abstracts,
49
50 and the literature meeting all the inclusion criteria will be independently assessed. In
51
52 case of disagreement, a third reviewer (ZLS) will be consulted. To measure interrater
53
54 agreement, the Kappa coefficients will be both calculated for the processes of titles/
55
56
57
58
59
60

1
2
3
4 abstract selection and full-text screening. The criteria for judging the scope of the
5
6 agreement between the evaluators are as follows: 0.00–0.20= slight agreement,
7
8 0.21–0.40= fair, 0.41–0.60= moderate, 0.61–0.80= substantial, and 0.81–1.00= almost
9
10 perfect agreement ⁶⁰. The plan of study screening and selection is available in Figure
11
12
13
14 2.

15 16 17 **Assessment of risk of bias**

18
19 The quality of the included studies will be assessed using NOS ⁵⁵. It is a tool mainly
20
21 used to evaluate the quality of case-control and cohort studies. The parameters
22
23 considered under each category are ① selection: case definition, representativeness
24
25 of the cases, selection of controls and definition of controls; ② comparability:
26
27 comparability of cases and controls based on the basis of the design or analysis; ③
28
29 exposure: ascertainment of exposure, the same method of ascertainment for cases and
30
31 controls, non-response rate. There are 1 to 2 stars in each category, with a maximum
32
33 of 9 stars for all. The number of stars is proportional to the mass of the study. The
34
35 number of stars is directly proportional to the quality of the study. The standard of
36
37 high quality will be NOS score ≥ 7 stars.
38
39

40
41 To ensure consistency in assessments, the two reviewers (HXG and HZ) will
42
43 independently evaluate the eligible literature according to NOS and will be
44
45 summarized in a table. When disagreements arise in the review, the third reviewer
46
47 (ZLS) cooperates with the team to reach a consensus.
48
49
50
51
52

53 54 55 **Data extraction**

56
57
58 Data from each eligible article will be extracted and compiled using a standardized
59
60

1
2
3
4 excel sheet. Items required for extraction will be obtained the PECOS steps. The
5
6 following data will be extracted for eligible studies: first author's surname, year of
7
8 publication, country, classification criteria for RA, number of cases and controls, age
9
10 and sex, disease duration, antibody positive of RA, 28-joint disease activity score,
11
12 medication, assessment methods of fecal microbiota, alterations in gut microbial
13
14 abundance, alpha-diversity indexes (OTUs, Shannon Index and Chao 1 Index) and
15
16 beta-diversity.
17
18
19
20
21
22

23 To conduct the meta-analysis, we involve trials that have available and sufficient data
24
25 to calculate the standardized mean difference (SMD) with 95% confidence interval
26
27 (CI) in RA patients and healthy controls in the analysis of the pooled data set. If
28
29 additional data or data transformations will be required for analysis, we will download
30
31 the publicly available raw data from online repositories or links provided in the
32
33 original publications. If there is no relevant data in the original literature, we will
34
35 acquire it after personal communication with the authors of the manuscripts. If the
36
37 authors do not reply, we will use Web Plot Digitizer (v.4.42) to digitize and extract
38
39 sufficient data from graphs and plots in the articles ^{49 61}.
40
41
42
43
44
45
46

47 To ensure the accuracy of the extracted data, we will randomly select two eligible
48
49 pieces of literature to be independently extracted by two reviewers (FQC and RZ).
50
51 Kappa will be applied to compare the consistency of data extraction from the two
52
53 literatures by the two reviewers. If there is an almost perfect agreement between the
54
55 two reviewers (Kappa value $\geq 80\%$), the remaining literature will extracted by one of
56
57 the two reviewers.
58
59
60

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 studies⁶²⁻⁶⁴. The included studies will be analyzed at the phylum or genus levels for consistency. 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 heterogeneity⁶⁵. 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 used⁵¹. 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 checklist⁶⁶. 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 bias⁶³. If funnel plots

1
2
3
4 present asymmetry, we will use Egger's test to statistically examination^{67 68}.
5

6 **Assessment of evidence quality**

7
8
9 We will conduct an appraisal of the quality of evidence by applying the Grading of
10 Recommendations Assessment, Development, and Evaluation (GRADE) framework
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

69. Two reviewers (YF and RZ) will assess 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/>).

31 **ETHICS AND DISSEMINATION**

32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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).

43 **PATIENT AND PUBLIC INVOLVEMENT**

44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

No patient or public involved.

51 **References**

1. Aletaha D, Smolen JS. Diagnosis and Management of Rheumatoid Arthritis: A Review. *JAMA* 2018;320(13):1360-72. doi: 10.1001/jama.2018.13103 [published Online First: 2018/10/05]
2. Alamanos YD, A. A. Epidemiology of adult rheumatoid arthritis. *Autoimmun Rev* 2005;4(3):130-6. doi: 10.1016/j.autrev.2004.09.002 [published Online First: 2005/04/13]
3. Gerlag DM, Norris JM, Tak PP. Towards prevention of autoantibody-positive rheumatoid arthritis: from lifestyle modification to preventive treatment. *Rheumatology (Oxford)*

- 2016;55(4):607-14. doi: 10.1093/rheumatology/kev347 [published Online First: 2015/09/17]
4. Taskforce ELAR. Rheuma Map A Research Roadmap to transform the lives of people with Rheumatic and Musculoskeletal Diseases. 2019
 5. Veale DJ, Orr C, Fearon U. Cellular and molecular perspectives in rheumatoid arthritis. *Semin Immunopathol* 2017;39(4):343-54. doi: 10.1007/s00281-017-0633-1 [published Online First: 2017/05/17]
 6. Zhou Y, Wang X, An Y, et al. Disability and health-related quality of life in Chinese patients with rheumatoid arthritis: A cross-sectional study. *Int J Rheum Dis* 2018;21(9):1709-15. doi: 10.1111/1756-185X.13345 [published Online First: 2018/10/23]
 7. Hu H, Luan L, Yang K, et al. Burden of rheumatoid arthritis from a societal perspective: A prevalence-based study on cost of this illness for patients in China. *Int J Rheum Dis* 2018;21(8):1572-80. doi: 10.1111/1756-185X.13028 [published Online First: 2017/02/18]
 8. Furneri G, Mantovani LG, Belisari A, et al. Systematic literature review on economic implications and pharmaco-economic issues of rheumatoid arthritis. *Clinical and experimental rheumatology* 2012;30(4 Suppl 73):S72-84. [published Online First: 2012/10/18]
 9. Shafrin J, Tebeka MG, Price K, et al. The Economic Burden of ACPA-Positive Status Among Patients with Rheumatoid Arthritis. *J Manag Care Spec Pharm* 2018;24(1):4-11. doi: 10.18553/jmcp.2017.17129 [published Online First: 2018/01/02]
 10. Alam J, Jantan I, Bukhari SNA. Rheumatoid arthritis: Recent advances on its etiology, role of cytokines and pharmacotherapy. *Biomed Pharmacother* 2017;92:615-33. doi: 10.1016/j.biopha.2017.05.055 [published Online First: 2017/06/06]
 11. Smolen JS, Landewe RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. *Ann Rheum Dis* 2020 doi: 10.1136/annrheumdis-2019-216655 [published Online First: 2020/01/24]
 12. Jakubovic BD, Donovan A, Webster PM, et al. Methotrexate-induced pulmonary toxicity. *Can Respir J* 2013;20(3):153-5. doi: 10.1155/2013/527912 [published Online First: 2013/06/14]
 13. Huang RY, Pan HD, Wu JQ, et al. Comparison of combination therapy with methotrexate and sinomenine or leflunomide for active rheumatoid arthritis: A randomized controlled clinical trial. *Phytomedicine* 2019;57:403-10. doi: 10.1016/j.phymed.2018.12.030 [published Online First: 2019/03/10]
 14. Lee EB, Fleischmann R, Hall S, et al. Tofacitinib versus methotrexate in rheumatoid arthritis. *N Engl J Med* 2014;370(25):2377-86. doi: 10.1056/NEJMoa1310476 [published Online First: 2014/06/19]
 15. Ramiro S, Sepriano A, Chatzidionysiou K, et al. Safety of synthetic and biological DMARDs: a systematic literature review informing the 2016 update of the EULAR recommendations for management of rheumatoid arthritis. *Ann Rheum Dis* 2017;76(6):1101-36. doi: 10.1136/annrheumdis-2016-210708 [published Online First: 2017/03/17]
 16. Karami J, Aslani S, Jamshidi A, et al. Genetic implications in the pathogenesis of rheumatoid arthritis; an updated review. *Gene* 2019;702:8-16. doi: 10.1016/j.gene.2019.03.033 [published Online First: 2019/03/25]
 17. Maeda Y, Takeda K. Host-microbiota interactions in rheumatoid arthritis. *Exp Mol Med* 2019;51(12):1-6. doi: 10.1038/s12276-019-0283-6 [published Online First: 2019/12/13]
 18. Konig MF. The microbiome in autoimmune rheumatic disease. *Best Pract Res Clin Rheumatol*

- 2020:101473. doi: 10.1016/j.berh.2019.101473 [published Online First: 2020/02/12]
19. Hager J, Bang H, Hagen M, et al. The Role of Dietary Fiber in Rheumatoid Arthritis Patients: A Feasibility Study. *Nutrients* 2019;11(10) doi: 10.3390/nu11102392 [published Online First: 2019/10/09]
20. Van de Wiele T, Van Praet JT, Marzorati M, et al. How the microbiota shapes rheumatic diseases. *Nat Rev Rheumatol* 2016;12(7):398-411. doi: 10.1038/nrrheum.2016.85 [published Online First: 2016/06/17]
21. Sun Y, Chen Q, Lin P, et al. Characteristics of Gut Microbiota in Patients With Rheumatoid Arthritis in Shanghai, China. *Frontiers in Cellular and Infection Microbiology* 2019;9:369. doi: 10.3389/fcimb.2019.00369 [published Online First: 2019/11/12]
22. Alpizar-Rodriguez D, Lesker TR, Gronow A, et al. *Prevotella copri* in individuals at risk for rheumatoid arthritis. *Ann Rheum Dis* 2019;78(5):590-93. doi: 10.1136/annrheumdis-2018-214514 [published Online First: 2019/02/15]
23. Nguyen Y, Mariette X, Salliot C, et al. Chronic diarrhoea and risk of rheumatoid arthritis: findings from the French E3N-EPIC Cohort Study. *Rheumatology (Oxford)* 2020 doi: 10.1093/rheumatology/keaa133 [published Online First: 2020/05/18]
24. Nemoto N, Takeda Y, Nara H, et al. Analysis of intestinal immunity and flora in a collagen-induced mouse arthritis model: differences during arthritis progression. *Int Immunol* 2020;32(1):49-56. doi: 10.1093/intimm/dxz058 [published Online First: 2019/09/29]
25. Zhang X, Zhang D, Jia H, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med* 2015;21(8):895-905. doi: 10.1038/nm.3914 [published Online First: 2015/07/28]
26. Chiang HI, Li JR, Liu CC, et al. An Association of Gut Microbiota with Different Phenotypes in Chinese Patients with Rheumatoid Arthritis. *J Clin Med* 2019;8(11) doi: 10.3390/jcm8111770 [published Online First: 2019/10/28]
27. Nayak RR, Alexander M, Stapleton-Grey K, et al. Perturbation of the human gut microbiome by a non-antibiotic drug contributes to the resolution of autoimmune disease. *bioRxiv* 2019 doi: 10.1101/600155
28. Jubair WK, Hendrickson JD, Severs EL, et al. Modulation of Inflammatory Arthritis in Mice by Gut Microbiota Through Mucosal Inflammation and Autoantibody Generation. *Arthritis Rheumatol* 2018;70(8):1220-33. doi: 10.1002/art.40490 [published Online First: 2018/03/14]
29. Mandel DR, Eichas K, Holmes J. *Bacillus coagulans*: a viable adjunct therapy for relieving symptoms of rheumatoid arthritis according to a randomized, controlled trial. *BMC complementary and alternative medicine* 2010;10:1. doi: 10.1186/1472-6882-10-1 [published Online First: 2010/01/14]
30. Vaghef-Mehrabany E, Alipour B, Homayouni-Rad A, et al. Probiotic supplementation improves inflammatory status in patients with rheumatoid arthritis. *Nutrition (Burbank, Los Angeles County, Calif)* 2014;30(4):430-5. doi: 10.1016/j.nut.2013.09.007 [published Online First: 2013/12/21]
31. So JS, Kwon HK, Lee CG, et al. *Lactobacillus casei* suppresses experimental arthritis by down-regulating T helper 1 effector functions. *Mol Immunol* 2008;45(9):2690-9. doi: 10.1016/j.molimm.2007.12.010 [published Online First: 2008/02/05]
32. Fan Z, Yang B, Ross RP, et al. Protective effects of *Bifidobacterium adolescentis* on collagen-induced arthritis in rats depend on timing of administration. *Food Funct*

- 2020;11(5):4499-511. doi: 10.1039/d0fo00077a [published Online First: 2020/05/10]
33. Lee YH. Causal association of gut microbiome on the risk of rheumatoid arthritis: a Mendelian randomisation study. *Ann Rheum Dis* 2020 doi: 10.1136/annrheumdis-2019-216747 [published Online First: 2020/01/12]
34. Uchiyama K, Naito Y, Takagi T. Intestinal microbiome as a novel therapeutic target for local and systemic inflammation. *Pharmacology & therapeutics* 2019;199:164-72. doi: 10.1016/j.pharmthera.2019.03.006 [published Online First: 2019/03/17]
35. Horta-Baas G, Sandoval-Cabrera A, Romero-Figueroa MJCr. Modification of Gut Microbiota in Inflammatory Arthritis: Highlights and Future Challenges. 2021;23(8):67. doi: 10.1007/s11926-021-01031-9
36. Gupta VK, Cunningham KY, Hur B, et al. Gut microbial determinants of clinically important improvement in patients with rheumatoid arthritis. *Genome Med* 2021;13(1):149. doi: 10.1186/s13073-021-00957-0 [published Online First: 2021/09/15]
37. Drago L. Prevotella Copri and Microbiota in Rheumatoid Arthritis: Fully Convincing Evidence? *J Clin Med* 2019;8(11) doi: 10.3390/jcm8111837 [published Online First: 2019/11/07]
38. Pianta A, Arvikar S, Strle K, et al. Evidence of the Immune Relevance of Prevotella copri, a Gut Microbe, in Patients With Rheumatoid Arthritis. *Arthritis Rheumatol* 2017;69(5):964-75. doi: 10.1002/art.40003 [published Online First: 2016/11/20]
39. Lorenzo D, GianVincenzo Z, Carlo Luca R, et al. Oral-Gut Microbiota and Arthritis: Is There an Evidence-Based Axis? *J Clin Med* 2019;8(10) doi: 10.3390/jcm8101753 [published Online First: 2019/10/28]
40. Maeda Y, Takeda K. Role of Gut Microbiota in Rheumatoid Arthritis. *J Clin Med* 2017;6(6) doi: 10.3390/jcm6060060 [published Online First: 2017/06/10]
41. Jeong Y, Kim JW, You HJ, et al. Gut Microbial Composition and Function Are Altered in Patients with Early Rheumatoid Arthritis. *J Clin Med* 2019;8(5) doi: 10.3390/jcm8050693 [published Online First: 2019/05/19]
42. Li X, Lu C, Fan D, et al. Human Umbilical Mesenchymal Stem Cells Display Therapeutic Potential in Rheumatoid Arthritis by Regulating Interactions Between Immunity and Gut Microbiota via the Aryl Hydrocarbon Receptor. *Frontiers in cell and developmental biology* 2020;8 doi: 10.3389/fcell.2020.00131
43. Chiang H-I, Li J-R, Liu C-C, et al. An Association of Gut Microbiota with Different Phenotypes in Chinese Patients with Rheumatoid Arthritis. *Journal of Clinical Medicine* 2019;8(11) doi: 10.3390/jcm8111770
44. Wu Y, Jiao N, Zhu R, et al. Identification of microbial markers across populations in early detection of colorectal cancer. *Nat Commun* 2021;12(1):3063. doi: 10.1038/s41467-021-23265-y [published Online First: 2021/05/26]
45. Najafi S, Abedini F, Azimzadeh Jamalkandi S, et al. The composition of lung microbiome in lung cancer: a systematic review and meta-analysis. *BMC microbiology* 2021;21(1):315. doi: 10.1186/s12866-021-02375-z [published Online First: 2021/11/13]
46. Ho NT, Li F, Lee-Sarwar KA, et al. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. *Nat Commun* 2018;9(1):4169. doi: 10.1038/s41467-018-06473-x [published Online First: 2018/10/12]
47. Shen T, Yue Y, He T, et al. The Association Between the Gut Microbiota and Parkinson's Disease, a Meta-Analysis. *Front Aging Neurosci* 2021;13:636545. doi: 10.3389/fnagi.2021.636545

- [published Online First: 2021/03/02]
48. Wirbel J, Pyl P, Kartal E, et al. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. 2019;25(4):679-89. doi: 10.1038/s41591-019-0406-6
 49. Nikolova V, Smith M, Hall L, et al. Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. 2021 doi: 10.1001/jamapsychiatry.2021.2573
 50. Iglesias-Vazquez L, Van Ginkel Riba G, Arija V, et al. Composition of Gut Microbiota in Children with Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Nutrients* 2020;12(3) doi: 10.3390/nu12030792 [published Online First: 2020/03/21]
 51. Xu M, Xu X, Li J, et al. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Front Psychiatry* 2019;10:473. doi: 10.3389/fpsy.2019.00473 [published Online First: 2019/08/14]
 52. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA. Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020). www.training.cochrane.org/handbook. www.training.cochrane.org/handbook.
 53. Moher D, Liberati, A., Tetzlaff, J., & Altman, D. G.; PRISMA Group.(2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Annals of Internal Medicine* 2009;151(4):264-69. doi: 10.7326/0003-4819-151-4-200908180-00135 %m 19622511
 54. Shamseer L, Moher D, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. 2015;349:g7647. doi: 10.1136/bmj.g7647 %J BMJ : British Medical Journal
 55. GA Wells BS, D O'Connell, J Peterson, V Welch, M Losos, P Tugwell,. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
 56. Morgan RL, Thayer KA, Bero L, et al. GRADE: Assessing the quality of evidence in environmental and occupational health. *Environment international* 2016;92-93:611-6. doi: 10.1016/j.envint.2016.01.004 [published Online First: 2016/02/02]
 57. Morgan RL, Whaley P, Thayer KA, et al. Identifying the PECO: A framework for formulating good questions to explore the association of environmental and other exposures with health outcomes. *Environment international* 2018;121(Pt 1):1027-31. doi: 10.1016/j.envint.2018.07.015 [published Online First: 2018/09/01]
 58. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010;62(9):2569-81. doi: 10.1002/art.27584 [published Online First: 2010/09/28]
 59. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31(3):315-24. doi: 10.1002/art.1780310302 [published Online First: 1988/03/01]
 60. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33(1):159-74. [published Online First: 1977/03/01]
 61. Safadi J, Quinton A, Lennox B, et al. Gut dysbiosis in severe mental illness and chronic fatigue: a novel trans-diagnostic construct? A systematic review and meta-analysis. 2021 doi: 10.1038/s41380-021-01032-1

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
62. Li F, Ye J, Shao C, et al. Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and Meta-analysis. *Lipids Health Dis* 2021;20(1):22. doi: 10.1186/s12944-021-01440-w [published Online First: 2021/02/28]
63. Kim KN, Yao Y, Ju SY. Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans with Obesity: A Systematic Review and Meta-Analysis. *Nutrients* 2019;11(10) doi: 10.3390/nu11102512 [published Online First: 2019/10/23]
64. Creedon AC, Hung ES, Berry SE, et al. Nuts and their Effect on Gut Microbiota, Gut Function and Symptoms in Adults: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. *Nutrients* 2020;12(8) doi: 10.3390/nu12082347 [published Online First: 2020/08/13]
65. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *Bmj* 2003;327(7414):557-60. doi: 10.1136/bmj.327.7414.557 [published Online First: 2003/09/06]
66. Campbell M, McKenzie J, Sowden A, et al. Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline. 2020;368:l6890. doi: 10.1136/bmj.l6890
67. Wang L, Alammari N, Singh R, et al. Gut Microbial Dysbiosis in the Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis of Case-Control Studies. *J Acad Nutr Diet* 2020;120(4):565-86. doi: 10.1016/j.jand.2019.05.015 [published Online First: 2019/09/02]
68. Ji R, Zhao X, Cao X, et al. Changes in gastric mucosal microbiota in gastric carcinogenesis: a systematic review protocol. 2021;11(3):e045810. doi: 10.1136/bmjopen-2020-045810
69. Bobbio E, Lingbrant M, Nwaru BI, et al. Inflammatory cardiomyopathies: short- and long-term outcomes after heart transplantation-a protocol for a systematic review and meta-analysis. *Heart Fail Rev* 2020;25(3):481-85. doi: 10.1007/s10741-020-09919-x [published Online First: 2020/01/15]

35
36
37
38
39
40
41
42
43
44
45
46
47

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.

48
49
50
51
52
53
54
55
56
57

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].

58
59
60

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	3839
#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 microbiotas'.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
#29	'gastric microbiomes'.ab,ti	9
#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 microbiotas'.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 microbiotas'.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

90x90mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

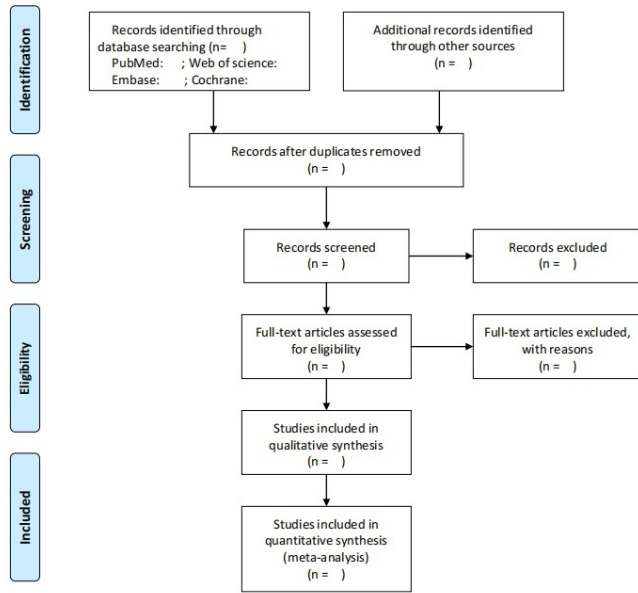


Figure 1 Plan of study screening and selection process

90x90mm (300 x 300 DPI)

BMJ Open

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

Journal:	<i>BMJ Open</i>
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

SCHOLARONE™
Manuscripts

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

Dan-wen Wang^{1#}, Xiang-tian Pang^{1#}, Heng Zhang¹, Hai-xia Gao¹, Yu-fei Leng², Feng-qin Chen¹, Rui Zhang¹, Yun Feng¹, Zhi-ling Sun^{1*}

1 School of Nursing, Nanjing University of Chinese Medicine, 138 Xianlin Road, Qixia District, Nanjing, Jiangsu Province 210023, China.

2 Animal Surgery Laboratory, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China

Dan-wen Wang and Xiang-tian Pang are joint first authors.

* Corresponding author: Zhiling Sun; Email: szl@njucm.edu.cn; telephone number: 13813892093; Address: Nursing College, Nanjing University of Chinese Medicine, 138 Xian Lin Road, Nanjing, Jiangsu Province 210023, China

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.

1
2
3
4 **Methods and analysis:** We will systematically search through PubMed, EMBASE,
5
6 Web of Science and Cochrane Library, as well as dissertations and conference
7
8 proceedings. The reference lists of all included studies will be also reviewed to
9
10 retrieve additional relevant studies. The case-control studies that reported either
11
12 the relative abundance of bacteria at the phylum or genus level or at least one
13
14 of the alpha-, beta-diversity indexes in both RA and healthy controls will be
15
16 included. Eligible studies will be screened independently by two reviewers according
17
18 to the inclusion criteria. The Newcastle-Ottawa Quality Assessment Scale will be used
19
20 to assess the quality of the included studies. Data extraction, qualitative and
21
22 quantitative analysis will be performed within the gut microbial dysbiosis in RA. The
23
24 expected outcomes will be the identification of the specific changes in
25
26 composition and diversity of the gut microbiota in patients with RA. The
27
28 quality of evidence will be assessed by the Grading of Recommendations Assessment,
29
30 Development, and Evaluation framework.
31
32
33
34
35
36
37
38

39
40 **Ethics and dissemination:** Ethical approval is unnecessary as this review does not
41
42 address the data and privacy of patients. The results will be published in a peer-
43
44 reviewed scientific journal and conference presentations.
45
46
47

48 **PROSPERO registration number:** CRD42021225229
49
50
51

52 53 **Strengths and limitations of this study**

54
55 This systematic review will identify the characteristic changes in the composition and
56
57 diversity of gut microbiota in patients with RA, a significant but controversial clinical
58
59
60

1
2
3
4 issue.

5
6 The relative abundances of phyla and/or genus levels in the gut microbiota
7
8 will be used in this meta-analysis.

9
10 The Web Plot Digitizer will be used to digitize and extract data from graphs and plots
11
12 may lead to biased results.

13
14 This systematic review will only include studies written in English, which may limit
15
16 available data or result in language bias.
17
18
19
20
21
22

23 24 **INTRODUCTION**

25
26
27 RA is a chronic disease characterized by persistent synovitis, inflammatory and
28
29 autoantibody changes ¹. The prevalence of RA is about 1% globally, and 1.02% in
30
31 China ². The prevalence of RA in women is 2-3 times higher than that in men ³. Delays
32
33 in diagnosis and treatment are associated with worse outcomes,
34
35 including irreversible joint destruction, disability and disease-related non-articular
36
37 outcomes such as reduced life span ^{4,5}. In China, 77.6% of RA patients had disabilities,
38
39 among which moderate and severe disabilities accounted for about 39%, seriously
40
41 affecting the quality of life of patients ⁶. The gradual deterioration of RA leads to a
42
43 sharp increase in the cost of the disease, which imposes a heavy societal and economic
44
45 burden on individuals and the country ⁷⁻⁹.

46
47
48 RA is a lifelong condition and currently no cure for most patients ^{10,11}. European League
49
50 Against Rheumatism (EULAR) and American College of Rheumatology (ACR)
51
52 recommend that the purpose of RA treatment should be to enable each patient to
53
54
55
56
57
58
59
60

1
2
3
4 achieve the goal of continuous remission or low disease activity ¹². Although the
5
6 prognosis of RA has improved with advances in diagnosis and treatment in recent
7
8 decades, the exact etiology and pathogenesis of RA are not fully understood. In order
9
10 to develop more effective treatment strategies for RA, it is essential to explore its
11
12 underlying etiology and pathogenesis.
13
14
15

16
17 Environmental factors are considered to play an important role in RA ¹³. The gut
18
19 microbiota is considered an important environmental factor in the development of RA
20
21 ¹⁴. Almost all studies on autoimmune rheumatic diseases show abnormal microbial
22
23 community structure (i.e. dysbiosis) ¹⁵. Dysbiosis not only affects the pro-inflammatory
24
25 and anti-inflammatory process of the intestinal mucosa, but also affects the distal joint
26
27 through the intestinal-joint axis ¹⁶⁻¹⁸. The studies have found dysbiosis in both RA
28
29 patients and high-risk individuals, indicating that the imbalance of intestinal flora could
30
31 have occurred before the onset of RA ^{14 19}. Dysbiosis has been involved in the
32
33 pathogenesis of RA in the decade before its diagnosis ²⁰. The intestinal flora imbalance
34
35 also appeared in the initial peak and relapse stage of RA ²¹. Dysbiosis is related to the
36
37 inflammatory response and disease activity of RA, which can be partially recovered by
38
39 effective treatment ²²⁻²⁴. As a first-line treatment for RA, methotrexate (MTX) may act
40
41 in part by modulating the human gut microbiota ²⁴. The results of animal experiments
42
43 suggest that interventions targeting intestinal microbiota may have the potential to
44
45 prevent RA in the preclinical stage ²⁵. Probiotics supplementation as adjunctive therapy
46
47 improves the inflammatory state of RA in human and animal studies ²⁶⁻²⁹. Therefore,
48
49 gut microbiota plays an important role in the development of RA, and may be a new
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 therapeutic target^{30 31}. Gut microbiome studies of RA are essential to elucidate etiology
5
6 and pathophysiological mechanisms and to develop potential therapeutic strategies.
7
8
9 Regulating the gut microbiota to slow the progression of the disease, especially in the
10
11 preclinical phase of RA, may be a promising approach for the treatment of RA in the
12
13 future^{32 33}.

14
15
16 Although numerous studies have shown that dysbiosis of the gut microbiome is a key
17
18 hallmark of RA, the distinct composition of the gut microbiome in RA patients remains
19
20 controversial. The abundance of *Prevotella* increased in patients with early RA, which
21
22 hurt the development and prognosis of RA^{14 34-37}. However, it has been reported that
23
24 the abundance of *Prevotella* did not significantly change in RA patients³⁸. Moreover,
25
26 *P. copri* and *P. histicola* of *Prevotella* have different effects on RA¹⁴. *Bacteroidetes*
27
28 were enriched in female patients with RA, while *Actinomycetes* and *Collinsella* were
29
30 enriched in healthy subjects³⁸. However, the abundance of *Bacteroides* and
31
32 *Bifidobacterium* was found to be reduced in RA patients and animal experiments^{23 39}.
33
34
35 It follows that the results of studies on the gut microbiota of RA patients are
36
37 contradictory. The identification of specific microbial profiles and patterns that may
38
39 contribute to the pathogenesis of RA remains a major challenge due to the inconsistent
40
41 results of studies on the gut microbiota. The conflicting results may stem from inter-
42
43 study batch effects, such as various biological factors influencing gut microbiome
44
45 composition, different data processing and analysis methods^{40 41}. The differences in
46
47 demographics of the study cohorts (e.g., sex, age, ethnicity, geography, and diet) also
48
49 have an important influence on the variability of the results of the gut microbiome study.
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 Through a quantitative review of the existing literature, the changes of RA gut
5
6 microbiota can be understood more clearly and comprehensively. Recently, several
7
8 meta-analyses of gut microbiota have identified specific microbial biomarkers
9
10 associated with disease ⁴²⁻⁴⁷. However, there has been no systematic review and meta-
11
12 analysis focusing on the characteristic dysbiosis of gut microbiota in RA to date.
13
14 Therefore, we will perform a systematic review and meta-analysis to identify
15
16 characteristic alterations in the gut microbiota of RA patients.
17
18
19
20
21

22 **OBJECTIVE**

23
24 The purpose of this protocol is to outline a systematic review and meta-analysis, which
25
26 evaluates the changes in the diversity of gut microbiota and the relative abundance of
27
28 bacterial phyla or genera in patients with RA.
29
30
31

32 **METHODS**

33 **Study design**

34
35 We plan to conduct a systematic review according to the Cochrane Handbook for
36
37 Systematic Reviews of Interventions Version 6.1 ⁴⁸, Preferred Reporting Items for
38
39 Systematic reviews and Meta-Analyses (PRISMA)⁴⁹, and PRISMA-Protocols
40
41 (PRISMA-P) 2015 ⁵⁰, as well as the Newcastle-Ottawa Quality Assessment Scale
42
43 (NOS)⁵¹. The PRISMA-P 2015 checklist is shown in Table 1. This protocol has been
44
45 registered at PROSPERO (registration number: CRD42021225229).
46
47
48
49
50
51

52 **Eligibility criteria**

53
54 The studies, written in English as eligible, will be selected and screened based on
55
56 PECOS steps (Population, Exposure, Comparator, Outcomes, and Study design) ^{52 53}.
57
58
59
60

1
2
3
4 The data items will be extracted as following:
5

6
7 ***Types of participants (P)***
8

9 The population of interest of the eligible studies should be adults (≥ 18 years old) with
10 met the diagnostic criteria (the ACR/EULAR 2010) for RA⁵⁴ or established RA (1987
11 classification criteria)⁵⁵ in the experimental group, the control group is a healthy
12 population.
13
14
15
16
17
18
19

20
21 ***Type of exposure (E)***
22

23 Trials were applied to assess the gut microbiota. Quantitative synthesis of microbiota
24 in fecal samples was performed by using metagenomic shotgun sequencing, 16s rRNA
25 sequencing techniques and/or real-time polymerase chain reaction (rt-PCR).
26
27
28
29

30
31 ***Comparison (C)***
32

33 Only healthy adults will be considered eligible for the control group.
34
35

36
37 ***Type of outcomes (O)***
38

39 The primary outcome of the study will be the identification of the composition of the
40 gut microbiome and the relative abundance of bacteria in RA. The secondary outcomes
41 will be considered: changes in the gut microbiota diversity (alpha-diversity, beta-
42 diversity), the effects of different gender and region on the relative abundance of gut
43 microbiota.
44
45
46
47
48
49
50

51
52 ***Type of studies (S)***
53

54 We will only include studies with the case-control design, written in English and
55 published in the original peer-reviewed journals. The animal studies, reviews, case
56
57
58
59
60

1
2
3
4 reports, and the full text unachieved will be excluded from the qualitative and
5
6 quantitative synthesis.
7

8 9 **Data sources and search strategies**

10
11 We conduct the search using the databases Embase, PubMed, Web of Science, and
12
13 Cochrane Library in the English language published up to September 2020. After
14
15 reading several documents, a search strategy combining medical subject terms (MeSH)
16
17 and free words was developed: ("Arthritis, Rheumatoid " OR Rheumatoid arthritis OR
18
19 RA) AND ("Gastrointestinal Microbiome " OR Gastrointestinal Microbiomes OR
20
21 Microbiome, Gastrointestinal OR Gut Microbiome OR Gut Microbiomes OR
22
23 Microbiome, Gut). The search strategy for the Embase database is shown in Figure 1.
24
25 To prevent the omission of the article, two researchers (DWW and XTP) will search
26
27 the above database independently. Using the snowball method, we manually search for
28
29 all references contained in the article.
30
31
32
33
34
35
36

37 38 **Screening procedures of eligible studies**

39
40 Once the search is complete, the literature will be managed using EndNote X9
41
42 (Clarivate Analytics (US) LLC) . Duplicates will be identified and deleted
43
44 according to Literature title. Then, the titles and abstracts of the literature will be
45
46 screened independently by two reviewers (XTP and YFL) according to the inclusion
47
48 criteria. Retrieval of the full text will be based on the eligible of titles and abstracts, and
49
50 the literature meeting all the inclusion criteria will be independently assessed. In case
51
52 of disagreement, a third reviewer (ZLS) will be consulted. To measure interrater
53
54 agreement, the Kappa coefficients will be both calculated for the processes of titles/
55
56
57
58
59
60

1
2
3
4 abstract selection and full-text screening. The criteria for judging the scope of the
5
6 agreement between the evaluators are as follows: 0.00–0.20= slight agreement, 0.21–
7
8 0.40= fair, 0.41–0.60= moderate, 0.61–0.80= substantial, and 0.81–1.00= almost
9
10 perfect agreement⁵⁶. The plan of study screening and selection is available in Figure 2.
11
12
13

14 **Assessment of risk of bias**

15
16
17 The quality of the included studies will be assessed using NOS⁵¹. It is a tool mainly
18
19 used to evaluate the quality of case-control and cohort studies. The parameters
20
21 considered under each category are ① selection: case definition, representativeness of
22
23 the cases, selection of controls and definition of controls; ② comparability:
24
25 comparability of cases and controls based on the basis of the design or analysis; ③
26
27 exposure: ascertainment of exposure, the same method of ascertainment for cases and
28
29 controls, non-response rate. There are 1 to 2 stars in each category, with a maximum of
30
31 9 stars for all. The number of stars is proportional to the mass of the study. The number
32
33 of stars is directly proportional to the quality of the study. The standard of high quality
34
35 will be NOS score ≥ 7 stars.
36
37
38
39
40
41
42

43 To ensure consistency in assessments, the two reviewers (HXG and HZ) will
44
45 independently evaluate the eligible literature according to NOS and will be summarized
46
47 in a table. When disagreements arise in the review, the third reviewer (ZLS) cooperates
48
49 with the team to reach a consensus.
50
51
52

53 **Data extraction**

54
55
56 Data from each eligible article will be extracted and compiled using a standardized
57
58 excel sheet. Items required for extraction will be obtained the PECOS steps. The
59
60

1
2
3
4 following data will be extracted for eligible studies: first author's surname, year of
5
6 publication, country, classification criteria for RA, number of cases and controls, age
7
8 and sex, disease duration, antibody positive of RA, 28-joint disease activity score,
9
10 medication, assessment methods of fecal microbiota, alterations in gut microbial
11
12 abundance, alpha-diversity indexes (OTUs, Shannon Index and Chao 1 Index) and beta-
13
14 diversity.
15
16
17
18
19

20 To conduct the meta-analysis, we involve trials that have available and sufficient data
21
22 to calculate the standardized mean difference (SMD) with 95% confidence interval (CI)
23
24 in RA patients and healthy controls in the analysis of the pooled data set. If additional
25
26 data or data transformations will be required for analysis, we will download the publicly
27
28 available raw data from online repositories or links provided in the original publications.
29
30
31 If there is no relevant data in the original literature, we will acquire it after personal
32
33 communication with the authors of the manuscripts. If the authors do not reply, we will
34
35 use Web Plot Digitizer (v.4.42) to digitize and extract sufficient data from graphs and
36
37 plots in the articles ^{45 57}.
38
39
40
41
42
43

44 To ensure the accuracy of the extracted data, we will randomly select two eligible pieces
45
46 of literature to be independently extracted by two reviewers (FQC and RZ). Kappa will
47
48 be applied to compare the consistency of data extraction from the two literatures by the
49
50 two reviewers. If there is an almost perfect agreement between the two reviewers
51
52 (Kappa value $\geq 80\%$), the remaining literature will be extracted by one of the two reviewers.
53
54
55

56 **Data synthesis and analysis**

57
58
59
60

1
2
3
4 When the number of studies for a single bacterium is five or more, we will conduct the
5
6 meta-analysis by R language Version 3.4.3 to compare the abundance level of gut
7
8 microbiota in RA patients with healthy controls. We will adopt SMD with 95% CI of
9
10 microbiota abundance as summary statistics when gut microbiota was detected by
11
12 different techniques in the included studies⁵⁸⁻⁶⁰. The included studies will be analyzed
13
14 at the phylum or genus levels for consistency. The forest plots will be used to visualize
15
16 the results. We will assess heterogeneity between studies using the Higgin I^2 statistic.
17
18 In relative terms, I^2 values are proportional to heterogeneity: I^2 values of 25%, 50%,
19
20 and 75% means low, moderate, and high heterogeneity⁶¹. Data analysis will be
21
22 performed by a random-effect model when there is substantial heterogeneity ($I^2 > 50%$);
23
24 otherwise, a fixed-effects model will be used⁴⁷. Additionally, we will conduct subgroup
25
26 analysis of different genders (male/female) and regions (east/west) included in the
27
28 studies.
29
30
31
32
33
34
35
36
37

38 If meta-analysis is not feasible, we will conduct narrative synthesis to summarize the
39
40 relevant evidence between RA and gut dysbiosis. The quantitative narrative synthesis
41
42 will be conducted according to the Synthesis Without Meta-analysis (SWiM) guideline
43
44 checklist⁶². In order to define the characteristics of the gut microbiota in RA, we will
45
46 perform compositional analysis based on the abundance, diversity, and specific
47
48 bacterial detection of gut microbiota in RA patients and healthy controls.
49
50
51

52 **Assessment of publication bias**

53
54
55 We will apply funnel plot and Egger's test to assess publication bias⁵⁹. If funnel plots
56
57 present asymmetry, we will use Egger's test to statistically examination^{63 64}.
58
59
60

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) framework⁶⁵.

Two reviewers (YF and RZ) will assess 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.

References

1. Aletaha D, Smolen JS. Diagnosis and Management of Rheumatoid Arthritis: A Review. *JAMA* 2018;320(13):1360-72. doi: 10.1001/jama.2018.13103 [published Online First: 2018/10/05]
2. Alamanos YD, A. A. Epidemiology of adult rheumatoid arthritis. *Autoimmun Rev* 2005;4(3):130-6. doi: 10.1016/j.autrev.2004.09.002 [published Online First: 2005/04/13]
3. Gerlag DM, Norris JM, Tak PP. Towards prevention of autoantibody-positive rheumatoid arthritis: from lifestyle modification to preventive treatment. *Rheumatology (Oxford)* 2016;55(4):607-14. doi: 10.1093/rheumatology/kev347 [published Online First: 2015/09/17]
4. Taskforce ELAR. Rheuma Map A Research Roadmap to transform the lives of people with Rheumatic and Musculoskeletal Diseases. 2019
5. Veale DJ, Orr C, Fearon U. Cellular and molecular perspectives in rheumatoid arthritis. *Semin*

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- Immunopathol* 2017;39(4):343-54. doi: 10.1007/s00281-017-0633-1 [published Online First: 2017/05/17]
6. Zhou Y, Wang X, An Y, et al. Disability and health-related quality of life in Chinese patients with rheumatoid arthritis: A cross-sectional study. *Int J Rheum Dis* 2018;21(9):1709-15. doi: 10.1111/1756-185X.13345 [published Online First: 2018/10/23]
7. Hu H, Luan L, Yang K, et al. Burden of rheumatoid arthritis from a societal perspective: A prevalence-based study on cost of this illness for patients in China. *Int J Rheum Dis* 2018;21(8):1572-80. doi: 10.1111/1756-185x.13028 [published Online First: 2017/02/18]
8. Furneri G, Mantovani LG, Belisari A, et al. Systematic literature review on economic implications and pharmaco-economic issues of rheumatoid arthritis. *Clinical and experimental rheumatology* 2012;30(4 Suppl 73):S72-84. [published Online First: 2012/10/18]
9. Shafrin J, Tebeka MG, Price K, et al. The Economic Burden of ACPA-Positive Status Among Patients with Rheumatoid Arthritis. *Journal of managed care & specialty pharmacy* 2018;24(1):4-11. doi: 10.18553/jmcp.2017.17129 [published Online First: 2018/01/02]
10. Smolen J, Landewé R, Bijlsma J, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. 2020;79(6):685-99. doi: 10.1136/annrheumdis-2019-216655
11. Alam J, Jantan I, Bukhari SNA. Rheumatoid arthritis: Recent advances on its etiology, role of cytokines and pharmacotherapy. *Biomed Pharmacother* 2017;92:615-33. doi: 10.1016/j.biopha.2017.05.055 [published Online First: 2017/06/06]
12. Smolen JS, Landewe RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. *Ann Rheum Dis* 2020 doi: 10.1136/annrheumdis-2019-216655 [published Online First: 2020/01/24]
13. Karami J, Aslani S, Jamshidi A, et al. Genetic implications in the pathogenesis of rheumatoid arthritis; an updated review. *Gene* 2019;702:8-16. doi: 10.1016/j.gene.2019.03.033 [published Online First: 2019/03/25]
14. Maeda Y, Takeda K. Host-microbiota interactions in rheumatoid arthritis. *Exp Mol Med* 2019;51(12):1-6. doi: 10.1038/s12276-019-0283-6 [published Online First: 2019/12/13]
15. Konig MF. The microbiome in autoimmune rheumatic disease. *Best Pract Res Clin Rheumatol* 2020;101473. doi: 10.1016/j.berh.2019.101473 [published Online First: 2020/02/12]
16. Hager J, Bang H, Hagen M, et al. The Role of Dietary Fiber in Rheumatoid Arthritis Patients: A Feasibility Study. *Nutrients* 2019;11(10) doi: 10.3390/nu11102392 [published Online First: 2019/10/09]
17. Van de Wiele T, Van Praet JT, Marzorati M, et al. How the microbiota shapes rheumatic diseases. *Nat Rev Rheumatol* 2016;12(7):398-411. doi: 10.1038/nrrheum.2016.85 [published Online First: 2016/06/17]
18. Sun Y, Chen Q, Lin P, et al. Characteristics of Gut Microbiota in Patients With Rheumatoid Arthritis in Shanghai, China. *Frontiers in Cellular and Infection Microbiology* 2019;9:369. doi: 10.3389/fcimb.2019.00369 [published Online First: 2019/11/12]
19. Alpizar-Rodriguez D, Lesker TR, Gronow A, et al. *Prevotella copri* in individuals at risk for rheumatoid arthritis. *Ann Rheum Dis* 2019;78(5):590-93. doi: 10.1136/annrheumdis-2018-214514 [published Online First: 2019/02/15]
20. Nguyen Y, Mariette X, Salliot C, et al. Chronic diarrhoea and risk of rheumatoid arthritis: findings

- 1
2
3 from the French E3N-EPIC Cohort Study. *Rheumatology (Oxford)* 2020 doi:
4 10.1093/rheumatology/keaa133 [published Online First: 2020/05/18]
5
6 21. Nemoto N, Takeda Y, Nara H, et al. Analysis of intestinal immunity and flora in a collagen-induced
7 mouse arthritis model: differences during arthritis progression. *Int Immunol* 2020;32(1):49-56.
8 doi: 10.1093/intimm/dxz058 [published Online First: 2019/09/29]
9
10 22. Zhang X, Zhang D, Jia H, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis
11 and partly normalized after treatment. *Nat Med* 2015;21(8):895-905. doi: 10.1038/nm.3914
12 [published Online First: 2015/07/28]
13
14 23. Chiang HI, Li JR, Liu CC, et al. An Association of Gut Microbiota with Different Phenotypes in Chinese
15 Patients with Rheumatoid Arthritis. *J Clin Med* 2019;8(11) doi: 10.3390/jcm8111770
16 [published Online First: 2019/10/28]
17
18 24. Nayak RR, Alexander M, Stapleton-Grey K, et al. Perturbation of the human gut microbiome by a
19 non-antibiotic drug contributes to the resolution of autoimmune disease. *bioRxiv* 2019 doi:
20 10.1101/600155
21
22 25. Jubair WK, Hendrickson JD, Severs EL, et al. Modulation of Inflammatory Arthritis in Mice by Gut
23 Microbiota Through Mucosal Inflammation and Autoantibody Generation. *Arthritis Rheumatol*
24 2018;70(8):1220-33. doi: 10.1002/art.40490 [published Online First: 2018/03/14]
25
26 26. Mandel DR, Eichas K, Holmes J. Bacillus coagulans: a viable adjunct therapy for relieving symptoms
27 of rheumatoid arthritis according to a randomized, controlled trial. *BMC Complement Altern*
28 *Med* 2010;10:1. doi: 10.1186/1472-6882-10-1 [published Online First: 2010/01/14]
29
30 27. Vaghef-Mehrabany E, Alipour B, Homayouni-Rad A, et al. Probiotic supplementation improves
31 inflammatory status in patients with rheumatoid arthritis. *Nutrition (Burbank, Los Angeles*
32 *County, Calif)* 2014;30(4):430-5. doi: 10.1016/j.nut.2013.09.007 [published Online First:
33 2013/12/21]
34
35 28. So JS, Kwon HK, Lee CG, et al. Lactobacillus casei suppresses experimental arthritis by down-
36 regulating T helper 1 effector functions. *Mol Immunol* 2008;45(9):2690-9. doi:
37 10.1016/j.molimm.2007.12.010 [published Online First: 2008/02/05]
38
39 29. Fan Z, Yang B, Ross RP, et al. Protective effects of Bifidobacterium adolescentis on collagen-induced
40 arthritis in rats depend on timing of administration. *Food Funct* 2020;11(5):4499-511. doi:
41 10.1039/d0fo00077a [published Online First: 2020/05/10]
42
43 30. Lee YH. Causal association of gut microbiome on the risk of rheumatoid arthritis: a Mendelian
44 randomisation study. *Ann Rheum Dis* 2020 doi: 10.1136/annrheumdis-2019-216747
45 [published Online First: 2020/01/12]
46
47 31. Uchiyama K, Naito Y, Takagi T. Intestinal microbiome as a novel therapeutic target for local and
48 systemic inflammation. *Pharmacol Ther* 2019;199:164-72. doi:
49 10.1016/j.pharmthera.2019.03.006 [published Online First: 2019/03/17]
50
51 32. Horta-Baas G, Sandoval-Cabrera A, Romero-Figueroa MJCr. Modification of Gut Microbiota in
52 Inflammatory Arthritis: Highlights and Future Challenges. 2021;23(8):67. doi: 10.1007/s11926-
53 021-01031-9
54
55 33. Gupta VK, Cunningham KY, Hur B, et al. Gut microbial determinants of clinically important
56 improvement in patients with rheumatoid arthritis. *Genome Med* 2021;13(1):149. doi:
57 10.1186/s13073-021-00957-0 [published Online First: 2021/09/15]
58
59 34. Drago L. Prevotella Copri and Microbiota in Rheumatoid Arthritis: Fully Convincing Evidence? *J Clin*
60 *Med* 2019;8(11) doi: 10.3390/jcm8111837 [published Online First: 2019/11/07]

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
35. Pianta A, Arvikar S, Strle K, et al. Evidence of the Immune Relevance of *Prevotella copri*, a Gut Microbe, in Patients With Rheumatoid Arthritis. *Arthritis Rheumatol* 2017;69(5):964-75. doi: 10.1002/art.40003 [published Online First: 2016/11/20]
36. Lorenzo D, GianVincenzo Z, Carlo Luca R, et al. Oral-Gut Microbiota and Arthritis: Is There an Evidence-Based Axis? *J Clin Med* 2019;8(10) doi: 10.3390/jcm8101753 [published Online First: 2019/10/28]
37. Maeda Y, Takeda K. Role of Gut Microbiota in Rheumatoid Arthritis. *J Clin Med* 2017;6(6) doi: 10.3390/jcm6060060 [published Online First: 2017/06/10]
38. Jeong Y, Kim JW, You HJ, et al. Gut Microbial Composition and Function Are Altered in Patients with Early Rheumatoid Arthritis. *J Clin Med* 2019;8(5) doi: 10.3390/jcm8050693 [published Online First: 2019/05/19]
39. Li X, Lu C, Fan D, et al. Human Umbilical Mesenchymal Stem Cells Display Therapeutic Potential in Rheumatoid Arthritis by Regulating Interactions Between Immunity and Gut Microbiota via the Aryl Hydrocarbon Receptor. *Frontiers in cell and developmental biology* 2020;8 doi: 10.3389/fcell.2020.00131
40. Wu Y, Jiao N, Zhu R, et al. Identification of microbial markers across populations in early detection of colorectal cancer. *Nat Commun* 2021;12(1):3063. doi: 10.1038/s41467-021-23265-y [published Online First: 2021/05/26]
41. Najafi S, Abedini F, Azimzadeh Jamalkandi S, et al. The composition of lung microbiome in lung cancer: a systematic review and meta-analysis. *BMC Microbiol* 2021;21(1):315. doi: 10.1186/s12866-021-02375-z [published Online First: 2021/11/13]
42. Ho NT, Li F, Lee-Sarwar KA, et al. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. *Nat Commun* 2018;9(1):4169. doi: 10.1038/s41467-018-06473-x [published Online First: 2018/10/12]
43. Shen T, Yue Y, He T, et al. The Association Between the Gut Microbiota and Parkinson's Disease, a Meta-Analysis. *Front Aging Neurosci* 2021;13:636545. doi: 10.3389/fnagi.2021.636545 [published Online First: 2021/03/02]
44. Wirbel J, Pyl P, Kartal E, et al. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. 2019;25(4):679-89. doi: 10.1038/s41591-019-0406-6
45. Nikolova V, Smith M, Hall L, et al. Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. 2021 doi: 10.1001/jamapsychiatry.2021.2573
46. Iglesias-Vazquez L, Van Ginkel Riba G, Arija V, et al. Composition of Gut Microbiota in Children with Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Nutrients* 2020;12(3) doi: 10.3390/nu12030792 [published Online First: 2020/03/21]
47. Xu M, Xu X, Li J, et al. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Front Psychiatry* 2019;10:473. doi: 10.3389/fpsy.2019.00473 [published Online First: 2019/08/14]
48. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA. Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020). www.training.cochrane.org/handbook. www.training.cochrane.org/handbook.
49. Moher D, Liberati A., Tetzlaff J., & Altman, D. G.; PRISMA Group.(2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Annals of Internal Medicine* 2009;151(4):264-69. doi: 10.7326/0003-4819-151-4-200908180-00135 %m

- 19622511
50. Shamseer L, Moher D, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. 2015;349:g7647. doi: 10.1136/bmj.g7647 %J BMJ : British Medical Journal
51. GA Wells BS, D O'Connell, J Peterson, V Welch, M Losos, P Tugwell,. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
52. Morgan RL, Thayer KA, Bero L, et al. GRADE: Assessing the quality of evidence in environmental and occupational health. *Environ Int* 2016;92-93:611-6. doi: 10.1016/j.envint.2016.01.004 [published Online First: 2016/02/02]
53. Morgan RL, Whaley P, Thayer KA, et al. Identifying the PECO: A framework for formulating good questions to explore the association of environmental and other exposures with health outcomes. *Environ Int* 2018;121(Pt 1):1027-31. doi: 10.1016/j.envint.2018.07.015 [published Online First: 2018/09/01]
54. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010;62(9):2569-81. doi: 10.1002/art.27584 [published Online First: 2010/09/28]
55. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31(3):315-24. doi: 10.1002/art.1780310302 [published Online First: 1988/03/01]
56. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33(1):159-74. [published Online First: 1977/03/01]
57. Safadi J, Quinton A, Lennox B, et al. Gut dysbiosis in severe mental illness and chronic fatigue: a novel trans-diagnostic construct? A systematic review and meta-analysis. 2021 doi: 10.1038/s41380-021-01032-1
58. Li F, Ye J, Shao C, et al. Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and Meta-analysis. *Lipids Health Dis* 2021;20(1):22. doi: 10.1186/s12944-021-01440-w [published Online First: 2021/02/28]
59. Kim KN, Yao Y, Ju SY. Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans with Obesity: A Systematic Review and Meta-Analysis. *Nutrients* 2019;11(10) doi: 10.3390/nu11102512 [published Online First: 2019/10/23]
60. Creedon AC, Hung ES, Berry SE, et al. Nuts and their Effect on Gut Microbiota, Gut Function and Symptoms in Adults: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. *Nutrients* 2020;12(8) doi: 10.3390/nu12082347 [published Online First: 2020/08/13]
61. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *Bmj* 2003;327(7414):557-60. doi: 10.1136/bmj.327.7414.557 [published Online First: 2003/09/06]
62. Campbell M, McKenzie J, Sowden A, et al. Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline. 2020;368:l6890. doi: 10.1136/bmj.l6890
63. Wang L, Alammari N, Singh R, et al. Gut Microbial Dysbiosis in the Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis of Case-Control Studies. *J Acad Nutr Diet* 2020;120(4):565-86. doi: 10.1016/j.jand.2019.05.015 [published Online First: 2019/09/02]
64. Ji R, Zhao X, Cao X, et al. Changes in gastric mucosal microbiota in gastric carcinogenesis: a systematic review protocol. 2021;11(3):e045810. doi: 10.1136/bmjopen-2020-045810

65. Bobbio E, Lingbrant M, Nwaru BI, et al. Inflammatory cardiomyopathies: short- and long-term outcomes after heart transplantation-a protocol for a systematic review and meta-analysis. *Heart Fail Rev* 2020;25(3):481-85. doi: 10.1007/s10741-020-09919-x [published Online First: 2020/01/15]

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:

1				
2				
3	Identification	1a	Identify the report as a protocol of a systematic review	1
4	Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
5				
6				
7	Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
8				
9	Authors:			
10	Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
11	Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	17
12				
13	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
14				
15	Support:			
16	Sources	5a	Indicate sources of financial or other support for the review	17
17	Sponsor	5b	Provide name for the review funder and/or sponsor	N/A
18	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
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				

INTRODUCTION

29	Rationale	6	Describe the rationale for the review in the context of what is already known	3-6
30	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

31	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
32	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
33	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
34				
35	Study records:			
36	Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8
37	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
38	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
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				
50				
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

1				
2				
3				
4	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
5				
6	Outcomes	13	List and define all outcomes for which data will be sought, including	
7	and		prioritization of main and additional outcomes, with rationale	11
8	prioritization			
9	Risk of bias in	14	Describe anticipated methods for assessing risk of bias of individual studies,	
10	individual studies		including whether this will be done at the outcome or study level, or both;	9
11			state how this information will be used in data synthesis	
12				
13	Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	10-11
14		15b	If data are appropriate for quantitative synthesis, describe planned summary	11
15			measures, methods of handling data and methods of combining data from	
16			studies, including any planned exploration of consistency (such as I^2 ,	
17			Kendall's τ)	
18		15c	Describe any proposed additional analyses (such as sensitivity or subgroup	11
19			analyses, meta-regression)	
20		15d	If quantitative synthesis is not appropriate, describe the type of summary	11
21			planned	
22				
23	Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias	11
24			across studies, selective reporting within studies)	
25				
26	Confidence	17	Describe how the strength of the body of evidence will be assessed (such as	
27	in		GRADE)	11-12
28	cumulative evidence			
29				
30				
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				
50				
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

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	3839
#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 microbiotas'.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
#29	'gastric microbiomes'.ab,ti	9
#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 microbiotas'.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 microbiotas'.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)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

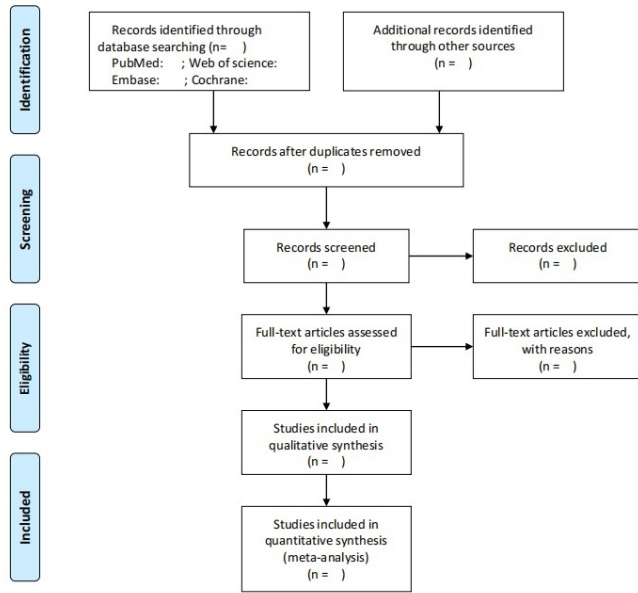


Figure 1 Plan of study screening and selection process

Figure2 Plan of study screening and selection process

90x90mm (300 x 300 DPI)