Systematic Review

The Gut Microbiome and Joint Microbiome Show Alterations in Patients With Knee Osteoarthritis Versus Controls: A Systematic Review

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Purpose: To assess the current scientific literature on the microbiome's relation with knee osteoarthritis (OA), with specific focuses on the gut microbiome–joint axis and joint microbiome–joint axis. **Methods:** A systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines; the PubMed, Embase, and Cochrane databases were searched for relevant English-language clinical studies on the gut and/or joint microbiomes' association with knee OA in humans. Bias was evaluated using the Methodological Index for Non-randomized Studies score. **Results:** Thirty-five thousand bacterial species comprise the gut microbiome; approximately 90% are members of the phyla Bacteroides and Firmicutes. Symbiosis between the gut microbiome and host under normal physiological conditions positively affects host growth, development, immunity, and longevity. Gut microbiome imbalance can negatively influence various physiological processes, including immune response, inflammation, metabolism, and joint health including the development of knee OA. In addition, next-generation gene sequencing suggests the presence of microorganisms in the synovial fluid of OA knees, and distinct microbiome profiles detected are presumed to play a role in the development of OA. Regarding the gut microbiome, consistent alterations in microbial composition between OA patients and controls are noted, in addition to several associations between certain gut bacteria and OA-related knee pain, patient-reported outcome measure performance, imaging findings, and changes in metabolic and inflammatory pathways. Regarding the joint microbiome, studies have revealed that increased levels of lipopolysaccharide and lipopolysaccharide-binding protein in synovial fluid are associated with activated macrophages—and are correlated with worsened osteophyte severity, joint space narrowing, and pain scores in knee OA patients. In addition, studies have shown various microbial composition differences in OA patients compared with controls, with certain joint microbes directly associated with OA pathogenesis, inflammation, and metabolic dysregulation. **Conclusions:** The gut microbiome–joint axis and joint microbiome show alterations in microbial composition between patients with OA and controls. These alterations are associated with perturbations of metabolic and inflammatory pathways, imaging findings, OA-related pain, and patient-reported outcome measure performance. **Level of Evidence:** Level III, systematic review of Level II and III studies.

Osteoarthritis (OA) is a prevalent and debilitating joint disorder, significantly impacting the quality of life of millions of persons globally and considered the most common degenerative joint disease.[1] Characterized by joint pain, stiffness, and a reduction in mobility, the etiology of OA is multifaceted, involving genetic, metabolic, and environmental factors.[2-4] Although OA is considered a degenerative noninflammatory disease, OA appears to be related to both a local low-grade inflammatory state intra-articularly and as an end-organ of a systemic inflammatory axis.[5,6]

Recent advances in microbiome research have sparked interest in the role of the gut microbiome in the development of systemic diseases.[7-10] The gut

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microbiome, a complex community of microorganisms residing in the gastrointestinal tract, is believed to play a crucial role in human health and disease.[11] Approximately 35,000 bacterial species comprise the gut microbiome; of these, approximately 90% are members of the phyla Bacteroides and Firmicutes.[12-14] Symbiosis between the gut microbiome and its host can be achieved under normal physiological conditions, in which a state of balance can positively affect the host's growth, development, immunity, and longevity.[15,16] Unfortunately, the gut microbiome can also negatively influence various physiological processes, including immune response, inflammation, and metabolism.[11] Dysbiosis, or imbalance in the gut microbiome, has been linked to a range of disorders and is assumed to indirectly affect the joint health.[5] In the context of OA, emerging evidence suggests a potential link between gut microbiome dysbiosis and the development or progression of OA.[15] With all this in mind, recent studies had endeavored to delve into understanding the relation between gut and joint microbiomes and the development of OA and general joint health.

Although extensive research efforts have been applied to deciphering the gut microbiome–joint axis, new studies are also reporting on the local intra-articular microbiome of the joint. Preliminary findings using next-generation sequencing (NGS) suggest the presence of microorganisms in the synovial fluid of the native osteoarthritic joint.[5,7,17,18] Moreover, the distinct knee microbiome profiles detected are presumed to play a role in the development of OA and periprosthetic joint infection.[18]

This systematic review aimed to critically assess the current scientific literature on the microbiome's relation with knee OA, with specific focuses on the gut microbiome–joint axis and joint microbiome-joint axis. We also aimed to examine recent evidence regarding the existence of the joint microbiome and its role in knee OA. We hypothesized that dysbiosis in the gut and/or joint microbiome would be significantly associated with the development and progression of knee OA.

Methods

A systematic review was conducted in adherence with the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines to identify Level I through IV studies examining the gut and/or joint microbiomes and their relations with knee OA. An extensive search was performed across the PubMed, Embase, and Cochrane databases using the following search terms: (gut microbiome OR gut OR microbiome) AND (arthritis OR osteoarthritis OR synovial OR synovitis) AND (knee OR joint).

The inclusion criteria included human clinical studies that investigated the association between the gut and/or joint microbiomes and knee OA, irrespective of intervention status, and were published in English. Articles that were excluded from consideration were those that exclusively addressed other forms of knee arthritis (e.g., rheumatoid, psoriatic, reactive, or juvenile arthritis or ankylosing spondylitis); were not available in English; exclusively involved nonhuman subjects; or were exclusively published as review articles, cadaveric studies, biomechanical studies, abstracts, or conference posters. After removal of duplicate studies, a thorough review of the studies' abstracts was conducted by 2 authors (K.M.J. and F.A.B.). Discrepancies were resolved by the senior author (B.J.C.). After the initial screening, articles were subjected to a full-text review. Any arising disagreements were again settled by the senior author. The final set of studies included in this systematic review was used to report the relation between the gut and/or joint microbiomes and knee OA.

Risk of Bias

Bias was assessed using the Methodological Index for Non-randomized Studies (MINORS) score.[19] This tool uses a numeric scale composed of 12 questions, with ideal scores of 16 for noncomparative studies and 24 for comparative studies.

Results

Study Selection and Characteristics

A comprehensive search across the 3 databases yielded a total of 1,843 studies. After removal of 477 duplicate studies, 1,366 studies remained for screening. Among these studies, 1,300 were excluded during abstract screening, leaving 66 studies for full-text review. Subsequently, 13 studies met the criteria for inclusion in this systematic review (Fig 1). Of the 13 included studies, 7 investigated the association between the gut microbiome and knee OA[20-26] whereas 6 examined the relation between the joint microbiome and knee OA.[17,27-31] Regarding the level of evidence, all 7 gut microbiome studies were classified as Level II[20-26]; 4 joint microbiome studies were also classified as Level II,[17,27,28,31] whereas 2 were categorized as Level III.[29,30] The results of the risk-of-bias assessment can be found in Figure 2, with Methodological Index for Non-randomized Studies (MINORS) scores ranging from 8 to 17 (mean, 13.1).

Gut Microbiome–Joint Axis

Demographic Characteristics. Of the 1,930 total patients from the 7 studies investigating the association between the gut microbiome and knee OA, 781 were male patients and 1,149 were female patients, with a weighted average

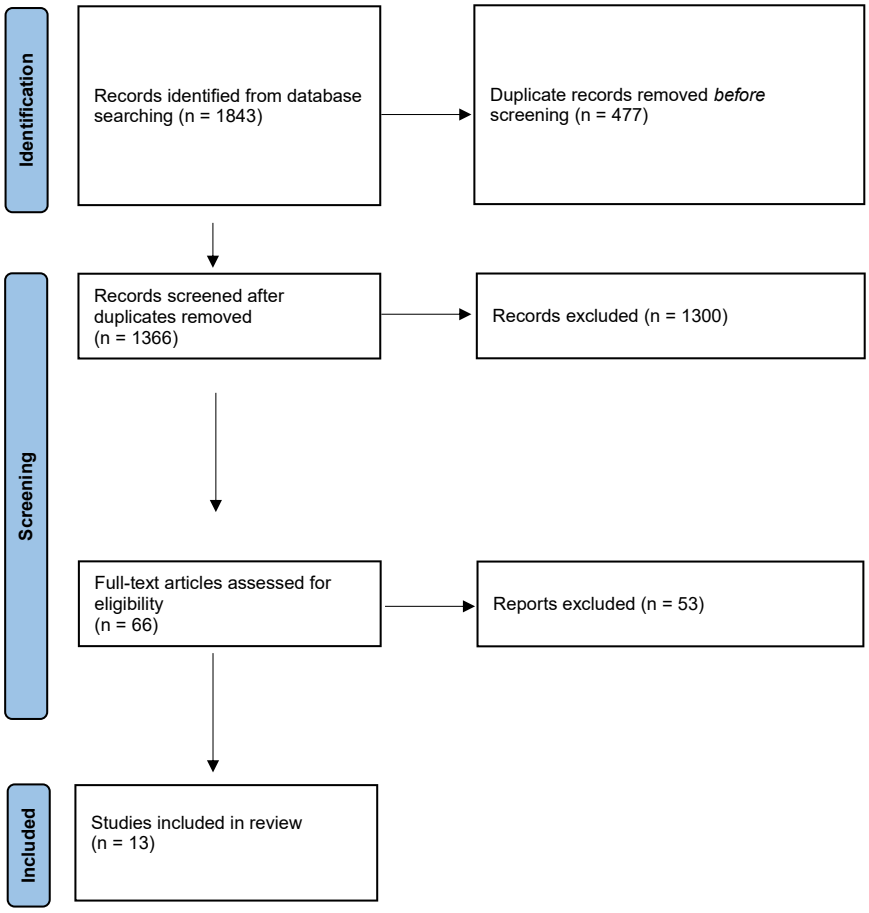

Fig 1. Preferred Reporting Items for Systematic Reviews and Meta-analyses study selection flow diagram.

age of 57.7 years and a weighted mean body mass index (BMI) of 27.1. Of these studies, 5 used RNA sequencing,[20,22-24,26] 1 used DNA sequencing,[25] and 1 used matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Table 1).[21]

Gut Microbiome Correlation to Knee OA. Coulson et al.[21] showed increased levels of *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Eubacterium*, *Lactobacillus*, *Bifidobacterium*, and *Clostridium* in OA patients compared with healthy controls. Liu et al.[22] observed differences in overall microbiome composition between OA patients and controls, with aerobic bacteria, gram-negative bacteria, and specifically, phylum Bacteroidota, class Bacteroidia, and order Bacteroidales displaying increased prevalence in OA patients, whereas *Prevotella copri* species, mobile elements, and gram-positive bacteria were more prevalent in controls. Liu et al.[22] also noted differences in metabolic pathways between the 2 groups, with decreases in DNA transcription, amino acid metabolism, adenosine triphosphate metabolism, and phospholipid metabolism in the OA group, whereas glucose metabolism, protein acetylation, and aspartate kinase activity were increased compared with controls.

Ramasamy et al.[23] reported higher bacterial diversity in stool samples from OA patients compared with controls, but the difference failed to reach the level of statistical significance. However, they identified significant enrichment of *Peptococcus*, *Shimwellia*, *Propionibacterium*, *Intestimonas*, and *Pavimonas* species in OA patients. Similarly, Wang et al.[24] showed no difference in overall gut microbiota diversity between OA patients and controls prior to electroacupuncture (EA) intervention. However, prior to intervention, OA patients receiving EA exhibited significantly elevated levels of *Blautia*, *Streptococcus*, and *Eubacterium hallii*, alongside significantly reduced levels of *Bacteroides* and *Agathobacter* compared with controls. Conversely, pre-intervention sham acupuncture (SA) OA patients showed significantly heightened levels of *Streptococcus* and *Anaerostipes*, with significantly decreased levels of *Bacteroides*, compared with controls.[24]

Wang et al.[25] noted decreases in genera *Agathobacter*, *Ruminococcus*, *Roseburia*, *Subdoligranulum*, and *Lactobacillus*, accompanied by increases in *Prevotella*,

	A clearly stated aim	Inclusion of consecutive patients	Prospective collection of data	Endpoints appropriate to the aim of the study	Unbiased assessment of the study endpoint	Follow-up period appropriate to the aim of the study	Loss to follow up less than 5%	Prospective calculation of study size	An adequate control group	Contemporary groups	Baseline equivalence of groups	Adequate statistical analyses	Total
Boer	2	1	2	1	2	2	0	0	N/A	N/A	N/A	N/A	10
Borsinger	2	2	2	1	1	2	2	2	N/A	N/A	N/A	N/A	14
Coulson	2	0	2	2	1	2	1	0	1	2	2	2	17
Dunn	2	0	2	2	0	2	2	0	N/A	N/A	N/A	N/A	10
Huang	2	0	2	2	1	2	0	0	N/A	N/A	N/A	N/A	9
Liu	2	2	2	2	0	2	0	0	2	0	1	1	14
Ramasamy	2	2	2	2	0	2	0	0	2	2	1	2	17
Siala	2	0	2	2	0	2	0	0	2	2	1	2	15
Tsai	2	0	1	2	0	2	0	0	2	2	1	2	14
Wang TQ	2	2	2	2	0	2	0	0	2	1	2	2	17
Wang X	1	0	2	2	0	2	0	0	2	1	2	1	13
Wang Z	1	0	1	2	0	2	0	0	2	0	2	2	12
Zhao	2	0	2	2	0	2	0	0	N/A	N/A	N/A	N/A	8

Fig 2. Results of Methodological Index for Non-Randomized Studies score bias grading for nonrandomized studies. Studies are scored as follows: 0, not reported (red); 1, reported but inadequate (yellow); or 2, reported and adequate (green). The maximum score for noncomparative studies is 16, and that for comparative studies is 24. (N/A, not applicable [noncomparative study].)

Clostridium, *Flavonifractor*, and *Klebsiella*, in OA patients compared with controls. Conversely, the control group exhibited increases in families Lactobacillaceae, Christensenellaceae, Clostridiaceae, and Acidaminococcaceae. Specific species also showed notable differences: *Bacteroides stercoris*, *Bacteroides vulgatus*, and *Bacteroides uniformis* were decreased in the OA group, whereas *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Streptococcus salivarius* were increased compared with controls.[25] Finally, Wang et al.[26] showed reduced diversity and richness of the microbiome in overweight OA patients compared with BMI-matched controls, with increases in *Gemmiger*, *Klebsiella*, *Akkermansia*, and *Lactobacillus* and decreases in *Bacteroides*, *Prevotella*, *Alistipes*, *Clostridium XI*, and *Parabacteroides* in overweight OA patients compared with BMI-matched controls.

Effects of Diet. Ramasamy et al.[23] additionally evaluated the effects of vitamin D deficiency on microbiome composition in patients with and without OA. Patients with vitamin D deficiency had significantly decreased abundances of *Paradoxostoma* species, class Clostridia, and genera *Megasphera*, *Bacteroides*, and *Subdogranulum*.

Clinical Findings and Interventions. Boer et al.[20] highlighted a positive correlation between higher OA-related knee pain and joint inflammation on magnetic resonance imaging (MRI) with a greater relative abundance of *Streptococcus*. Wang et al.[24] showed significant correlations between specific bacterial taxa and clinical parameters; *Bacteroides*, *Agathobacter*, *Faecalibacterium*, and *Roseburia* were negatively correlated with pain scores and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) total score, whereas *Streptococcus* and *Enterococcus* showed positive correlations with pain scores. *Streptococcus* was also positively associated with the WOMAC total score, whereas *E hallii*, *Blautia*, and *Anaerostipes* exhibited positive correlations with 12-Item Short Form Health Survey (SF-12) physiological and psychological scores. Wang et al.[24] additionally showed that, at 8 weeks after intervention, pain scores and WOMAC total scores improved more in the EA group than in the SA group and that the number of significantly different genera between OA patients and controls was less after EA compared with SA. Finally, Coulson et al.[21] showed significant improvement in all OA outcome measures in OA patients receiving green-lipped mussel extract or glucosamine sulfate at 12 weeks after intervention, including improvement in the Lequesne index and WOMAC total, pain, stiffness, and physical function scores.

Joint Microbiome–Joint Axis

Demographic Characteristics. Of the 184 patients in the 6 studies examining the relation between the joint microbiome and knee OA, 58 were male patients and

Table 1. Level of Evidence, Country, OA Location, Subject Number, Sex, BMI, Age, Detection Method, Sample, and Main Findings for All 7 Gut Microbiome Studies Included in Systematic Review

Authors (Year)	Level of Evidence	Country	OA Location	Subjects, n	M/F Sex, n	BMI	Age, yr	Detection Method	Sample	Main Findings
Boer et al.[20] (2019)	II	The Netherlands	Knee	Radiographic OA: 124 Radiographic control: 817	606/821	27.5 ± 4.5	56.9 ± 5.9	RNA sequencing	Stool	The gut microbiome—in particular, a greater abundance of *Streptococcus*—was found to be associated with higher knee WOMAC scores for pain and joint inflammation on MRI.
Coulson et al.[21] (2013)	II	Australia	Knee	38 (21 OA patients treated with GLM and 17 OA patients treated with GLS)	GLM: 5/16 GLS: 5/12	GLM: 31.3 ± 6.1 GLS: 30.2 ± 4.8	GLM: 56.7 ± 8.9 GLS: 60 ± 8.6	Microflex MALDI-TOF mass spectrometry (Bruker)	Stool	Compared with control data, both OA groups at pre-intervention baseline showed increased levels of *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Eubacterium*, *Lactobacillus*, *Bifidobacterium*, and *Clostridium*. At 12 weeks after intervention, both the GLM and GLS groups showed significant improvement in all OA outcome measures, including improvement in the Lequesne index and WOMAC total, pain, stiffness, and physical function scores.
Liu et al.[22] (2022)	II	China	Knee	OA: 40 Control: 40	OA: 10/30 Control: 10/30	OA: 25.71 (20.4-40.0) Control: 20.20 (17.85-23.94)	OA: 68 (53-80) Control: 26 (24-28)	RNA sequencing	Stool	The study showed a significant difference in the overall composition of the microbiome, with phylum Bacteroidota, class Bacteroidia, and order Bacteroidales being more

(continued)

Table 1. Continued

Authors (Year)	Level of Evidence	Country	OA Location	Subjects, n	M/F Sex, n	BMI	Age, yr	Detection Method	Sample	Main Findings
										prevalent in OA patients, whereas *Prevotella copri* species was more prevalent in control patients. In the OA group, DNA transcription, amino acid metabolism, ATP metabolism, and phospholipid metabolism significantly decreased, whereas glucose metabolism, protein acetylation, and aspartate kinase activity significantly increased, compared with controls. Aerobic ($P = .003$) and gram-negative ($P < .001$) bacteria were more prevalent in the OA group, whereas mobile elements ($P = .001$) and gram-positive ($P < .001$) bacteria were more prevalent in the control group.
Ramasamy et al.[23] (2021)	II	India	Knee	NVDD OA: 4 NVDD control: 6 VDD OA: 7 VDD control: 7	NVDD OA: 1/3 NVDD control: 6/0 VDD OA: 1/6 VDD control: 7/0	NVDD OA: 27.5 ± 2.8 NVDD control: 22.9 ± 2.6 VDD OA: 28.2 ± 2.7 VDD control: 28.9 ± 2.6	NVDD OA: 50 ± 9.7 NVDD control: 37.7 ± 12.7 VDD OA: 52 ± 7.2 VDD control: 44 ± 8.1	RNA sequencing	Stool	NVDD OA samples had overall higher bacterial diversity than NVDD control samples, but the difference failed to reach the level of statistical significance. However, NVDD OA patients showed significantly more enrichment with *Peptococcus*, *Shimwellia*, *Propionibacterium*, *Intestimonas*, and *Pavimonas* species

(continued)

Table 1. Continued

Authors (Year)	Level of Evidence	Country	OA Location	Subjects, n	M/F Sex, n	BMI	Age, yr	Detection Method	Sample	Main Findings
										compared with NVDD controls. VDD patients had significantly less decreased abundances of *Megasphera* (genus level), *Bacteroides* (genus level), *Subdogranulum* (genus level), *Paradoxostoma* (species level), and Clostridia (class level).
Wang et al.[24] (2021)	II	China	Knee	OA treated with EA: 30 OA treated with SA: 30 Control: 30	EA: 11/19 SA: 10/20 Control: 11/19	EA: 26.04 ± 2.92 SA: 25.86 ± 4.02 Control: 24.58 ± 3.01	EA: 64.73 ± 5.39 SA: 66.10 ± 7.42 Control: 63.67 ± 6.94	RNA sequencing	Stool	No difference in overall gut microbiota diversity was found between the control group and either OA group before intervention. However, pre-intervention EA patients showed significantly higher amounts of *Blautia*, *Streptococcus*, and *Eubacterium hallii* and significantly lower levels of *Bacteroides* and *Agathobacter* compared with controls. Meanwhile, pre-intervention SA patients showed significantly higher amounts of *Streptococcus* and *Anaerostipes* and significantly lower levels of *Bacteroides* compared with controls. *Bacteroides*, *Agathobacter*, *Faecalibacterium*, and *Roseburia* were negatively

(continued)

Table 1. Continued

Authors (Year)	Level of Evidence	Country	OA Location	Subjects, n	M/F Sex, n	BMI	Age, yr	Detection Method	Sample	Main Findings
										correlated with pain scores and WOMAC total scores. *Streptococcus* and *Enterococcus* were positively correlated with pain scores, and *Streptococcus* was also positively correlated with WOMAC total scores. *E hallii*, *Blautia*, and *Anaerostipes* were positively correlated with SF-12 physiological and psychological scores. At 8 weeks after intervention, pain scores and WOMAC total scores improved more in the EA group than in the SA group. The number of significantly different genera between OA patients and controls was less after EA compared with SA.
Wang et al.[25] (2023)	II	China	Knee	OA: 32 Control: 57	OA: 9/23 Control: 24/33	OA: 24.24 Control: 23.79	OA: 68.2 ± 3.5 Control: 62 ± 2.4	DNA sequencing	Stool	The abundances of genera *Agathobacter*, *Ruminococcus*, *Roseburia*, *Subdoligranulum*, and *Lactobacillus* were significantly decreased in the OA group compared with the control group. The abundances of genera *Prevotella*, *Clostridium*, *Flavonifractor*, and *Klebsiella* were significantly increased in the OA

(continued)

Table 1. Continued

Authors (Year)	Level of Evidence	Country	OA Location	Subjects, n	M/F Sex, n	BMI	Age, yr	Detection Method	Sample	Main Findings
										group. The families Lactobacillaceae, Christensenellaceae, Clostridiaceae, and Acidaminococcaceae were significantly increased in the control group. The abundances of species *Bacteroides stercoris*, *Bacteroides vulgatus*, and *Bacteroides uniformis* were significantly decreased in the OA group, and the abundances of species *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Streptococcus salivarius* were significantly increased in the OA group.
Wang et al.[26] (2021)	II	China	Knee	OA: 86 Control: 96	OA: 25/61 Control: 40/56	25-30	OA: 62 (50-72) Control: 64 (50-76)	RNA sequencing	Stool	In overweight patients with OA, diversity and richness of the microbiome were reduced compared with controls. *Gemmiger*, *Klebsiella*, *Akkermansia*, and *Lactobacillus* were significantly increased in the gut microbiome of OA patients compared with controls, whereas *Bacteroides*, *Prevotella*, *Alistipes*, *Clostridium XI*, and *Parabacteroides* were significantly decreased in OA patients compared with controls.

NOTE. Continuous variables were directly extracted from the included studies and are presented as mean, mean ± standard deviation, mean (range), or range depending on the respective study.

ATP, adenosine triphosphate; BMI, body mass index; EA, electroacupuncture; F, female; GLM, green-lipped mussel extract; GLS, glucosamine sulfate; M, male; MALDI-TOF, matrix-assisted laser desorption/ionization time of flight; MRI, magnetic resonance imaging; NVDD, non−vitamin D−deficient; OA, osteoarthritis; SA, sham acupuncture; SF-12, 12-Item Short Form Health Survey; VDD, vitamin D−deficient; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

68 were female patients, whereas the sex of 58 patients was not identified. The weighted average age was 60.2 years, and the weighted mean BMI was 31.7. Of these studies, 1 used NGS,[17] 1 used EndoZyme assay (bioMérieux),[30] 2 used RNA sequencing,[27,31] and 2 used DNA sequencing (Table 2).[28,29]

Joint Microbiome Correlation to Knee OA. Huang et al.[30] showed that increased synovial fluid levels of lipopolysaccharide (LPS) and lipopolysaccharide-binding protein (LBP) were associated with an abundance of activated macrophages in the synovium. Borsinger et al.[17] revealed positive NGS results in 3.8% of OA knees, with *Cutibacterium acnes* being the predominant organism detected. There was no significant difference in OA severity between groups with positive and negative NGS results, as indicated by Kellgren-Lawrence grading, C-reactive protein level, white blood cell count, or percentage of polymorphonuclear leukocytes.[17] Conversely, Siala et al.[28] found the presence of bacterial DNA in the synovial fluid samples of 50% of OA patients (3 of 6), with *Stenotrophomonas maltophilia* and *Shigella* species being the 2 most frequently identified species.

Tsai et al.[29] found 43 microbes (almost half *Pseudomonas* species) that were more abundant in OA patients compared with controls, with 9 directly associated with OA pathogenesis and playing a role in increased inflammation-induced extracellular matrix remodeling and decreased cellular communication critical for joint and immune function. Meanwhile, Dunn et al.[27] reported 41 bacterial clades that differed between OA knee cartilage samples and cadaveric control cartilage samples, with levels of class Clostridium and phylum Bacteroidetes being increased in control cartilage samples, whereas class Betaproteobacteria and order Burkholderiales were increased in OA cartilage samples.

Finally, Zhao et al.[31] analyzed synovial fluid and tissue of OA patients and identified Proteobacteria, Bacteroidetes, and Firmicutes as the most abundant phyla in both sample types. They also found microbial genera present in synovial fluid showed a negative correlation with various metabolism-related pathways, including amino acid, carbohydrate, energy, coenzyme, vitamin, terpenoid, ketone, and nucleotide metabolism, while showing a positive correlation with transcription factors. Conversely, microbial genera found in synovial tissue exhibited a positive correlation with metabolic-related pathways, such as amino acid, carbohydrate, energy, coenzyme, vitamin, terpenoid, ketone, and nucleotide metabolism, as well as cytoskeletal proteins and drug metabolism—related enzymes. Additionally, they displayed a negative correlation with degradation and metabolism-related pathways of foreign substances, phenylalanine metabolism, dicarboxylic acid metabolism, unsaturated fatty acid biosynthesis, and protein kinase.

Effects of BMI. Huang et al.[30] found that increased serum LPS and LBP concentrations were both significantly associated with increased BMI whereas synovial fluid LPS and LBP concentrations showed no significant associations with BMI.

Clinical Findings and Interventions. Huang et al.[30] showed that increased LPS levels were associated with increased osteophyte severity, increased joint space narrowing severity, and higher WOMAC total scores whereas increased LBP levels were associated with higher self-reported knee pain scores.

Discussion

The synthesis of current literature on the gut and joint microbiomes in knee OA reveals significant insights into the potential role of microbial dysbiosis in disease pathogenesis. The collective evidence from studies examining the gut microbiome—joint axis revealed consistent alterations in microbial composition between OA patients and controls. Particularly noteworthy were the associations found between higher levels of *Streptococcus* and increased knee pain and joint inflammation, as well as the associations of other gut bacteria with OA pain parameters and perturbations of notable metabolic pathways, indicating a potential pathogenic role for certain bacterial taxa. Furthermore, investigations into the joint microbiome revealed microbial presence in articular cartilage, synovial fluid, and synovial tissue, with discernible associations between microbial abundance, inflammation markers, disease severity, and various metabolic pathways, shedding light on the complex interplay between microbiota and OA pathology.

In the analysis of gut microbiota composition, certain bacteria exhibited significant variations in prevalence among OA patients across multiple studies. Notably, *Streptococcus* species were found to be markedly more prevalent in OA patients across 4 studies.[20,21,24,25] Boer et al.[20] and Wang et al.[24] showed associations between its abundance and higher OA-related knee pain, WOMAC total scores, and joint inflammation on MRI. This could be elucidated by the role of *Streptococcus* in precluding local and systemic inflammation through LPS-induced macrophage activation[32] or through the LBP-enhanced formation of CD14-LPS complexes during the macrophage response.[33,34] This aligns well with prior literature that has shown increased CD14 levels, a marker of proinflammatory macrophages, in the synovium of OA patients.[35] Consequently, an increase in the prevalence of *Streptococcus* species could dysregulate the inflammatory milieu of the joint, potentially exacerbating inflammatory responses in OA. Conversely, *Bacteroides* species were found to be more abundant in control patients across 3 studies and were

Table 2. Level of Evidence, Country, OA Location, Subject Number, Sex, BMI, Age, Detection Method, Sample, and Main Findings for All 6 Joint Microbiome Studies Included in Systematic Review

Authors (Year)	Level of Evidence	Country	OA Location	Subjects, n	M/F Sex, n	BMI	Age, yr	Detection Method	Sample	Main Findings
Borsinger et al.[17] (2023)	II	United States	Knee	40 (80 total knees, comprising 50 knees undergoing arthroplasty and 30 control knees; all with KL grade 2-4)	18/22	32.4 (22.4-49.5)	67 (41-84)	NGS	Synovial fluid	Positive NGS results were found in 3 of 80 knees (3.8%). *Cutibacterium acnes* was most common. No difference in OA status was observed between the positive NGS group (100% with KL grade 4) and negative NGS group (83.8% with KL grade 4, 8.1% with KL grade 3, and 8.1% with KL grade 2) ($P = .751$). No difference in C-reactive protein level, WBC count, or percentage of PMNs was observed between the positive and negative NGS groups.
Dunn et al.[27] (2020)	II	United States	Knee	OA: 21 Control (cadaveric cartilage): 10	OA: 9/12 Control: 6/4	OA: 34 ± 1 Control: 30 ± 3	OA: 59 ± 2 Control: 68 ± 4	RNA sequencing	Cartilage	A total of 41 clades were found to be different between OA knees and control knees. Notably, class Clostridium and phylum Bacteroidetes were both increased in control knees compared with OA knees, whereas class Betaproteobacteria and order Burkholderiales were both increased in OA knees compared with control knees.
Huang et al.[30] (2016)	III	China and United States	Knee	25 (31 knees, all with KL grade 1-4)	7/18	29.2 ± 4.8	62.4 ± 15.8	EndoZyme assay	Synovial fluid	Synovial fluid levels of LPS and LBP were associated with the abundance of activated macrophages in the synovium. Synovial fluid LPS levels were associated with osteophyte severity, joint space narrowing severity, and higher WOMAC total scores. Synovial fluid LBP levels were associated with higher self-reported knee pain scores. Serum LPS and LBP concentrations were both significantly correlated with BMI, whereas synovial fluid LPS and LBP concentrations were not.

(continued)

Table 2. Continued

Authors (Year)	Level of Evidence	Country	OA Location	Subjects, n	M/F Sex, n	BMI	Age, yr	Detection Method	Sample	Main Findings
Siala et al.[28] (2009)	II	Tunisia and France	Knee	6 (OA patients with knee effusion)	5/1	NA	58 (44-70)	DNA sequencing	Synovial fluid	The presence of bacterial DNA was found in the synovial fluid samples of 3 of 6 OA patients (50%). *Stenotrophomonas maltophilia* and *Shigella* species were the 2 most frequently identified bacterial DNA sequences.
Tsai et al.[29] (2020)	III	United States	Knee	OA: 14 Control: 10	OA: 6/8 Control: 7/3	NA	OA: 50.2 (19-69) Control: 37.8 (22-63)	RNA sequencing	Synovial fluid	A total of 43 microbes were found to be more abundant in OA patients, with almost half being *Pseudomonas* species. Of these 43 microbes, 9 were found to be correlated with OA pathogenesis and to play a role in (1) increased inflammation-induced extracellular matrix remodeling and (2) decreased cellular communication essential for joint function and immune function.
Zhao et al.[31] (2018)	II	China	Knee	58 (42 OA patients with synovial fluid drawn and 16 OA patients with synovial tissue drawn)	NA	NA	NA	RNA sequencing	Synovial fluid and tissue	The most abundant phyla identified were Proteobacteria (55.1% of synovial tissue samples and 39.1% of synovial fluid samples), Bacteroidetes (20.4% of synovial tissue samples and 29.4% of synovial fluid samples), and Firmicutes (17.0% of synovial tissue samples and 24.0% of synovial fluid samples). Microbial genera present in synovial fluid exhibited a negative correlation with metabolism-related pathways, including amino acid, carbohydrate, energy, coenzyme, vitamin, terpenoid, ketone, and nucleotide metabolism, while showing a positive correlation with transcription factors. In

(continued)

Table 2. Continued

Authors (Year)	Level of Evidence	Country	OA Location	Subjects, n	M/F Sex, n	BMI	Age, yr	Detection Method	Sample	Main Findings
										contrast, microbial genera found in synovial tissue displayed a positive correlation with metabolic-related pathways, such as amino acid, carbohydrate, energy, coenzyme, vitamin, terpenoid, ketone, and nucleotide metabolism, as well as cytoskeletal proteins and drug metabolism −related enzymes, while exhibiting a negative correlation with degradation and metabolism-related pathways of foreign substances, phenylalanine metabolism, dicarboxylic acid metabolism, unsaturated fatty acid biosynthesis, and protein kinase.

NOTE. Continuous variables were directly extracted from the included studies and are presented as mean ± standard deviation or mean (range) depending on the respective study.

BMI, body mass index; F, female; KL, Kellgren-Lawrence; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; M, male; NA, not available (data not reported by study); NGS, next-generation sequencing; OA, osteoarthritis; PMN, polymorphonuclear cell; WBC, white blood cell; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

negatively correlated with pain scores and WOMAC total scores.[24-26] This may be attributed to the production of short-chain fatty acids, such as acetate and propionate, by bacteria within the *Bacteroidetes* genus. These fatty acids are known to induce and regulate cell differentiation of regulatory T cells, which plays a role in suppressing both chronic and acute inflammation.[36,37] As such, decreased levels of *Bacteroides* species in OA patients may lead to compromised anti-inflammatory responses and subsequently worse patient-reported outcome measures. Overall, the distinct associative variations in bacterial abundances between OA and control patients underscore the need for further exploration of their potential contributory or protective roles in OA pathogenesis.

Despite a similarly small number of studies analyzing the relation between the joint microbiome and OA, the findings of this review reveal a complex microbial landscape within OA-affected knees. Borsinger et al.[17] showed positive NGS results in the synovial fluid of 3.8% of arthritic knees, with no significant difference in OA severity or immunologic parameters compared with arthritic knees with negative NGS results. However, they acknowledged that the low percentage of positive NGS results may be attributed to their study's small sample size and changes in NGS protocols compared with other studies, thereby making it more challenging to achieve positive NGS results.[17] Meanwhile, Siala et al.[28] discovered the presence of bacterial DNA in 50% of OA patients' synovial fluid samples (albeit with a small sample size as well), Dunn et al.[27] reported 41 bacterial clades that differed between OA cartilage and control cartilage samples, and Tsai et al.[29] identified 43 unique microbes when comparing OA and control synovial fluid samples, with nearly half being *Pseudomonas* species that were more abundant in OA patients. Among these, Tsai et al. identified 9 species, including 5 *Pseudomonas* species, directly associated with OA through captured immune signatures and dysregulation of 2 main pathways: increased inflammation-induced extracellular matrix remodeling and decreased cellular communication critical for joint and immune function. Notably, 6 of 9 species were associated with activated mast cell infiltration, which may potentially play a role in OA progression.[29,38] In a separate study illustrating ties between the immunoregulatory system and the joint microbiota, Huang et al.[30] showed that microbial byproducts such as increased LPS and LBP levels were associated with an abundance of activated macrophages in the synovium, which may contribute to pathologic changes in the joint. These findings align with additional results from Huang et al. showing that increased LPS levels were associated with increased osteophyte severity, increased joint space narrowing severity, and higher WOMAC total scores, whereas increased LBP levels were associated with higher self-reported knee pain scores. Similar to discussions concerning the gut microbiome–joint axis, the observed associations are significant and offer insight into potential mechanistic pathways linking joint microbial presence to OA. However, further evidence is necessary to establish causality definitively.

A prospective multicenter study published by Goswami et al.[39] aimed to explore the microbial composition within the joints of patients with OA. They enrolled 113 patients undergoing knee or hip arthroplasty and performed DNA extraction, followed by microbial 16S-ribosomal RNA sequencing of synovial fluid, tissue, and swab specimens. They found the most abundant genera to be *Escherichia*, *Cutibacterium*, *Staphylococcus*, *Acinetobacter*, and *Pseudomonas*. They also reported that hospital origin was associated with certain strains and a prior corticosteroid injection in the past 6 months was correlated with elevated abundances of several lineages. This study was not included in the current systematic review because it included both knee and hip OA patients and the outcomes were reported in such a manner that it did not allow extraction of data regarding the knee OA patients only. However, the findings of this well-designed study are of importance and may likely still be highly specific to the knee joint microbiome. Additional well-designed studies have also shown a potential clinical association between gut dysbiosis and permeability and the development of periprosthetic joint infection.[40,41]

A small number of studies within this review additionally investigated the associations and effects that diet,[23] BMI,[30] and clinical interventions targeting the microbiome[21,24] may have on microbiome composition and/or clinical outcomes of patients with knee OA. Ramasamy et al.[23] reported significant associations between vitamin D deficiency and altered gut microbiome composition, highlighting the potential influence of micronutrient status on OA disease management. Huang et al.[30] observed notable associations between increased BMI and increased serum concentrations of LPS and LBP while observing no associations between BMI and synovial concentrations of LPS or LBP, suggesting potential differences in systemic versus localized effects of obesity on the microbiome in the setting of OA. Wang et al.[24] showed improved pain scores in OA patients and reduced differences in gut microbial composition between OA patients and controls after EA, suggesting a potential role for acupuncture in modulating the gut microbiome and, consequently, potentially alleviating OA symptoms. Similarly, the findings of Coulson et al.[21] regarding the efficacy of green-lipped mussel extract and glucosamine sulfate in improving OA outcome measures highlight the potential importance of dietary supplements as adjunct therapies for OA management. The collective insights

from these studies, in conjunction with the concurrent findings of this review, challenge the traditional view of OA as solely a mechanical disorder, underscoring the importance of considering the gut microbiome and local joint microbiome in both understanding disease heterogeneity and identifying potential targets for therapeutic interventions in knee OA.

To enhance the current literature, future studies should look to establish possible causal relations between highly associated bacteria and OA on both metabolic and clinical levels, including patient-reported outcome measures, radiographic findings, and MRI results. Additionally, the microbiome shows promise as a target for innovative OA treatment strategies. Interventions addressing dysbiosis in OA patients, such as probiotics, dietary modifications, acupuncture, and fecal transplantation, merit further investigation. However, standardized formulations and numerous large randomized controlled trials are needed to conclusively determine the efficacy of these interventions, as well as any future microbiome-targeted therapy, for treating OA. Furthermore, thorough investigation into the intricate interplay among gut dysbiosis, inflammation, and OA severity through longitudinal and interventional studies is critical for developing precision therapies and fully understanding the complex relation between microbial communities and OA pathogenesis.

Limitations

This study has several limitations. First, the limited availability of literature, coupled with potential selection bias, restricted both the quantity and quality of studies eligible for inclusion. Furthermore, the considerable heterogeneity observed across included studies, characterized by variations in sample sizes, methodologies, and participant demographic characteristics, may limit the generalizability of findings. Additionally, each included study may have been susceptible to confounding variables and biases, potentially influencing its respective results. Finally, publication bias and language limitations may have affected the comprehensiveness of the review, potentially omitting relevant studies and introducing language-based biases. Despite these challenges, this systematic review serves as a valuable synthesis of existing literature, providing insights and highlighting areas for further investigation.

Conclusions

The gut microbiome–joint axis and joint microbiome show alterations in microbial composition between patients with OA and controls. These alterations are associated with perturbations of metabolic and inflammatory pathways, imaging findings, OA-related pain, and patient-reported outcome measure performance.

Disclosures

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