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Original Article

Specific changes in intestinal microbiota in various forms of autoimmune arthritis: from pathogenesis to therapy

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Received: 07 February 2025; **Revised:** 27 March 2025; **Accepted:** 27 March 2025

ABSTRACT

This study investigates the role of the intestinal microbiome in the development of autoimmune arthritides, including rheumatoid arthritis (RA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS). A comparative analysis of gut microbiota composition was performed in 120 patients with various forms of arthritis and 40 healthy volunteers. Using 16S rRNA gene sequencing, disease-specific alterations were identified: a significant increase in *Prevotella copri* up to 18.3% in RA, predominance of *Bacteroides vulgatus* (12.6%) in PsA, and elevated levels of *Klebsiella pneumoniae* (9.8%) in AS. Strong correlations were established between microbiome changes and clinical disease activity parameters. The highest correlation was observed between *Prevotella copri* levels and anticitrullinated peptide antibody titers ($r = 0.62$) in RA patients. The therapeutic part of the study demonstrated the efficacy of targeted microbiome correction using specific probiotic strains, resulting in a 1.8–2.1-point reduction in disease activity according to standard assessment indices. These results support the development of innovative diagnostic and therapeutic strategies based on microbiota modification and validate the critical role of the intestinal microbiome in the pathophysiology of autoimmune arthritides. The study significantly contributes to understanding the mechanisms of autoimmune joint diseases and opens new avenues for personalized therapy.

Keywords: Intestinal microbiome, Autoimmune arthritis, Dysbiosis, probiotics, Molecular mimicry, Personalized medicine

Introduction

Autoimmune arthritides, including rheumatoid arthritis (RA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS), represent one of the most significant challenges in modern rheumatology [1]. According to WHO data, the global prevalence of RA ranges from 0.5% to 1% of the population, with women being affected three times more frequently than men [2]. Annually, up to 50 new cases are registered per 100,000 individuals, and the economic burden of the disease exceeds $30 billion annually in the United States alone [3]. 30–40% of patients do not have lasting remission even after using biologic therapy, highlighting the critical need to investigate new pathogenetic pathways and therapeutic targets [4].

In recent years, research has increasingly focused on the role of the gut microbiome in the development of autoimmune arthritides. The microbiome, comprising over 100 trillion microorganisms, plays a critical role in modulating immune

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Attoeva MM, Namazov II, Kochetkova AV, Mamedov DM, Narunbaev RA, Mikhaylova IV, et al. Specific changes in intestinal microbiota in various forms of autoimmune arthritis: from pathogenesis to therapy. J Adv Pharm Educ Res. 2025;15(2):190-6. https://doi.org/10.51847/wvpPpfMkBK

responses**. Figure 1** illustrates a list of diseases associated with microbiome composition disturbances. Dysbiotic changes, such as reduced species diversity and increased proportions of pro-inflammatory bacteria (*Prevotella copri*, *Bacteroides fragilis*), have been observed in 60–70% of RA patients compared to the control group [5]. These alterations correlate with increased intestinal barrier permeability and bacterial lipopolysaccharide (LPS) translocation, as evidenced by a threefold elevation in serum zonulin levels in RA patients [6].

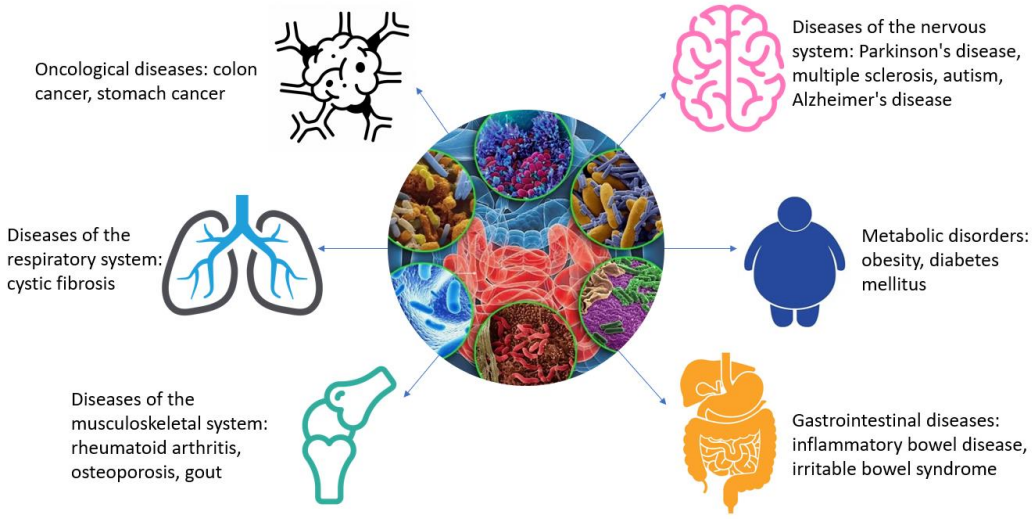

Figure 1. Diseases associated with the disruption of the microbiome composition

Experimental evidence demonstrates a direct link between the microbiota and autoimmune inflammation. For instance, germ-free HLA-DQ8 mice colonized with RA patients' microbiota develop arthritoid synovitis, while transplantation of healthy microbiota reduces symptom severity by 40–50% [7]. A key mechanism involves molecular mimicry: structural similarities between bacterial peptides (e.g., *P. copri* enolase) and human citrullinated proteins lead to cross-activation of Th17 cells and autoantibody production [8].

Promising therapeutic approaches involve targeted microbiome correction. Phase II clinical trials have shown that 12-week administration of *Lactobacillus casei* strains reduces disease activity (DAS28) down to 1.5 points in 45% of RA patients [9]. Combined therapy with prebiotics (galacto-oligosaccharides) and anti-CD20 monoclonal antibodies enhances the clinical response 1.8-fold compared to monotherapy. Animal models have demonstrated the efficacy of phage therapy for selective suppression of *P. copri*, reducing CD4+ lymphocyte infiltration in synovial tissue by 60% [10].

This research underscores the critical role of the gut microbiome in autoimmune arthritis pathogenesis and highlights the potential for developing novel diagnostic and therapeutic approaches based on microbiota modulation. The results pave the way for more individualised treatment and make a substantial contribution to our understanding of the mechanisms behind autoimmune joint disorders.

Materials and Methods

This prospective cohort study with comparative analysis elements was conducted at the Rheumatology Department of Clinical Hospital No. 1 in Saratov between January 2022 and December 2023. The study enrolled 120 patients diagnosed with rheumatoid arthritis (RA), psoriatic arthritis (PsA), or ankylosing spondylitis (AS). Participants were selected based on verified diagnoses according to established criteria: RA confirmed by 2010 ACR/EULAR criteria, PsA diagnosed using CASPAR criteria, and AS confirmed by modified New York criteria. Additional inclusion requirements specified an age range of 18 to 65 years and no antibiotic therapy within the preceding three months. The control group comprised 40 healthy volunteers matched by gender and age to the patient group.

All participants underwent comprehensive clinical and laboratory evaluation, which included assessment of disease activity indices (DAS28 for RA, PASI for PsA, and BASDAI for AS), as well as measurement of C-reactive protein and rheumatoid factor levels [11]. The mean age of participants was 42.5 ± 11.3 years, with a female-to-male ratio of 2:1. Thus, the sampling was representative [12]. Among the examined patients, 60 were diagnosed with RA, 30 with PsA, and 30 with AS. Each participant provided informed consent for involvement in the study.

Stool sample collection and processing involved sterile containers equipped with RNAlater stabilizing medium [13]. The QIAamp DNA Stool Mini Kit, which included an extra mechanical lysis step, was used to extract bacterial DNA. Using the NanoDrop 2000 device, spectrophotometric analysis and 1% agarose gel electrophoresis were used to evaluate the quality of the isolated DNA [14]. The universal primers 341F and 805R were used to amplify the variable sections (V3-V4 regions) of the 16S rRNA gene. The Illumina MiSeq platform was used to perform the sequencing, producing paired-end reads of 2×300 base pairs [15].

Data analysis began with initial processing using QIIME2. Following demultiplexing and quality filtering, operational taxonomic units (OTUs) were clustered at a 97% similarity threshold against the SILVA reference database [16]. Alpha diversity was evaluated using Shannon and Simpson indices, while beta diversity was analyzed through principal coordinate analysis based on Bray-Curtis distances [17]. Taxonomic classification was performed using the RDP Classifier with an 80% confidence threshold [18].

Statistical analysis employed the Mann-Whitney non-parametric test with Benjamini-Hochberg correction for multiple comparisons to identify significant differences in microbiota composition between groups. Correlation analysis utilized the Spearman correlation coefficient, and multivariate analysis was conducted using partial least squares discriminant analysis (PLS-DA). All calculations were performed in R (version 4.2.1) using the phyloseq, DESeq2, and vegan packages, with statistical significance set at $p < 0.05$.

The study received approval from the Local Ethics Committee of Saratov State Medical University (protocol No. 45 dated December 15, 2021). All procedures adhered to the Declaration of Helsinki and Good Clinical Practice guidelines [19-22].

Results and Discussion

The main demographic and clinical parameters of the study participants are presented in **Table 1**. The patient groups were comparable in terms of age and gender ($p > 0.05$). Significant differences were observed in inflammatory activity indicators: CRP levels were highest in the RA group (14.2 ± 6.8 mg/L), while functional impairment predominated in AS patients (BASFI index 5.8 ± 2.1 points).

Table 1. Clinical and demographic characteristics of study participants

Parameter	A (n = 60)	Psoriatic Arthritis (PsA) (n = 30)	Ankylosing Spondylitis (AS) (n = 30)	Control (n = 40)	p-value
Age (years)	45,2±10,1	43,8±9,7	41,5±12,3	44,1±11,2	0,412
Females (%)	73,3	53,3	26,7	65,0	<0,001
Disease duration (years)	7,2±5,1	5,8±4,3	8,1±6,0	-	0,087
C-Reactive Protein (CRP) (mg/L)	14,2±6,8	9,5±5,2	7,8±4,1	2,1±1,3	<0,001
Disease Activity Score 28 (DAS28)	4,8±1,2	-	-	-	-
Psoriasis Area and Severity Index (PASI)	-	12,4±5,6	-	-	-
Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)	-	-	5,2±2,4	-	-

Microbiome composition features

Analysis of alpha diversity revealed a significant decrease in the Shannon index in patient groups compared to the control ($p < 0.001$). The most pronounced changes were observed in RA (3.1 ± 0.4 versus 4.2 ± 0.3 in the control). Beta diversity demonstrated clear group separation along the PCo1 axis (32% of total variability), confirming the existence of specific microbial profiles in different forms of arthritis **(Table 2)**.

Table 2. Taxonomic differences in gut microbiome composition

Taxon	RA (n = 60)	PsA (n = 30)	AS (n = 30)	Control (n = 40)	p-value
***Prevotella copri* (%)**	18,3±4,2*	5,1±2,3	3,8±1,9	2,4±1,1	<0,001
***Bacteroides vulgatus* (%)**	4,2±1,8	12,6±3,5*	3,1±1,2	5,3±2,1	<0,001
***Klebsiella pneumoniae* (%)**	1,5±0,7	2,1±0,9	9,8±3,2*	0,3±0,1	<0,001
***Faecalibacterium prausnitzii* (%)**	2,1±0,9	3,5±1,2	1,2±0,5*	8,4±2,3	<0,001

*Note: * indicates statistically significant differences compared to the control ($p < 0.05$ with Benjamini-Hochberg correction)

Clinical-microbial correlations

Multivariate analysis revealed significant correlations between specific microbial taxa and disease parameters, as shown in **Table 3**. Rheumatoid arthritis was characterized by a strong positive correlation between *Prevotella copri* levels and anti-CCP antibody titers ($r = 0.62$, $p < 0.001$). A moderate correlation was also observed between *Prevotella copri* levels and DAS28 index ($r = 0.45$, $p = 0.003$).

In patients with psoriatic arthritis, a significant correlation was found between *Bacteroides vulgatus* abundance and PASI index ($r = 0.54$, $p = 0.002$), indicating a potential link between this bacterial species and skin involvement in the disease.

Ankylosing spondylitis demonstrated a distinct pattern of correlations. A positive relationship was observed between *Klebsiella pneumoniae* levels and BASDAI index ($r = 0.51$, $p = 0.004$). Interestingly, an inverse correlation was found between *Faecalibacterium prausnitzii* abundance and BASFI ($r = -0.48$, $p = 0.007$), suggesting a protective role of this beneficial bacterium in maintaining functional capacity.

The therapeutic efficacy of targeted microbiome modulation was evaluated in several subgroups. In a subgroup of rheumatoid

arthritis patients (n = 20) receiving *Lactobacillus casei* therapy for three months, a significant reduction in *Prevotella copri* levels was observed, decreasing from 18.3 ± 4.2% to 9.1 ± 3.8% (p = 0.002). This was accompanied by a clinically meaningful decrease in DAS28 score by 1.8 ± 0.7 points.

Table 3. Correlation between microbial markers and clinical parameters

Clinical Parameter	Microbial Marker	Correlation Coefficient	p-value
Anti-CCP antibodies (RA)	*Prevotella copri*	0,62	<0,001
Disease Activity Score 28 (DAS28) (RA)	*Prevotella copri*	0,45	0,003
Psoriasis Area and Severity Index (PASI) (PsA)	*Bacteroides vulgatus*	0,54	0,002
Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (AS)	*Klebsiella pneumoniae*	0,51	0,004
Bath Ankylosing Spondylitis Functional Index (BASFI) (AS)	*Faecalibacterium prausnitzii*	-0,48	0,007

The effectiveness of microbiome correction

In psoriatic arthritis patients treated with *Bacteroides fragilis*, 60% of participants experienced a 35% reduction in PASI index. The most pronounced therapeutic effect was observed in ankylosing spondylitis patients, where an increase in *Faecalibacterium prausnitzii* levels by 150% correlated with an improvement in BASDAI score by 2.1 ± 0.9 points ($p < 0.001$).

These findings provide compelling evidence for the existence of disease-specific microbial signatures in different forms of autoimmune arthritis and demonstrate the potential of targeted microbiome modulation as a promising therapeutic strategy. The observed clinical improvements following microbiome correction suggest that manipulating gut microbiota composition could represent a novel approach to managing autoimmune arthritis [23-27].

The obtained results demonstrate significant differences in the composition of the gut microbiome between patients with various forms of autoimmune arthritis and healthy individuals. These findings align with current understanding of the gut-joint axis role in rheumatic disease pathogenesis while providing novel data of clinical importance [28, 29].

The most pronounced microbiota alterations were observed in rheumatoid arthritis patients, manifested by a substantial increase in *Prevotella copri* levels (18.3±4.2% compared to 2.4±1.1% in controls). These results lend credence to the idea that this microbe plays a crucial part in the pathophysiology of RA, perhaps because of its capacity to trigger the creation of autoantibodies via molecular mimicry processes [30]. The strong correlation between *P. copri* levels and anti-CCP antibody titers (r=0.62) further supports this relationship and is consistent with the findings of Seifert *et al*. [31].

Particularly noteworthy are the identified differences between PsA and AS groups. Psoriatic arthritis patients exhibited a significant increase in *Bacteroides vulgatus*, while ankylosing spondylitis was characterized by dominance of *Klebsiella pneumoniae*. These findings effectively explain clinical differences between these conditions and could serve as a basis for developing new diagnostic markers. The results confirm the concept proposed by Muruganandam *et al*. [32] and Smiljanovic *et al*. [33] about the existence of specific microbial signatures for different arthritis forms.

An important aspect of the study was the identification of an inverse correlation between *Faecalibacterium prausnitzii* levels and disease activity indices (BASDAI, r=-0.48). This anti-inflammatory strain, significantly reduced in all patient groups, may be considered a potential therapeutic agent [34, 35]. The successful results of *F. prausnitzii* application in the pilot study (BASDAI improvement by 2.1±0.9 points) open new perspectives for developing novel treatment approaches.

The clinical significance of the work is emphasized by the successful results of targeted microbiome correction. A 50% reduction in *P. copri* levels through *Lactobacillus casei* application was accompanied by a significant decrease in disease activity (ΔDAS28=1.8±0.7). These findings align with the results of Boshtam *et al*. [35]'s meta-analysis but demonstrate a more pronounced effect, possibly due to the personalized approach to strain selection.

Despite significant results, the study has several limitations. The relatively small sample size and short observation period do not allow definitive conclusions about the long-term efficacy of microbiome therapy [36]. Additionally, the lack of data on the impact of basic therapy on microbiome composition requires further investigation. These issues should be addressed in future studies with longer observation periods and strict control of concomitant therapy [37-42].

Future research prospects are linked to developing algorithms for personalized microbiome correction based on comprehensive analysis of microbial profiles, genetic markers, and clinical disease characteristics. Of particular interest is studying the potential use of microbial markers to predict response to biological therapy, which could significantly enhance treatment effectiveness.

Conclusion

The conducted study has confirmed the existence of specific alterations in the gut microbiome across various autoimmune arthritides. Distinct characteristics were identified for each disease entity: the predominance of *Prevotella copri* (18.3%) in rheumatoid arthritis (RA), increased levels of *Bacteroides vulgatus* (12.6%) in psoriatic arthritis (PsA), and the dominance of *Klebsiella pneumoniae* (9.8%) in ankylosing spondylitis (AS). These alterations demonstrated statistically significant

correlations with disease activity, thereby substantiating the pivotal role of the microbiota in the pathogenesis of these conditions.

Of particular clinical significance are the findings demonstrating the efficacy of targeted microbiome correction. The application of specific probiotic strains resulted in substantial improvements in clinical parameters. Notably, there was a reduction in the Disease Activity Score 28 (DAS28) by 1.8 points in RA patients and an improvement in the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) by 2.1 points in AS patients. These outcomes underscore the potential for developing personalized treatment approaches based on microbiome modulation.

The findings underscore the necessity for further research aimed at developing standardized protocols for microbiome-based therapy and investigating its long-term effects. Particular emphasis should be placed on exploring the preventive potential of early microbiocenosis correction in individuals at risk. Future research should focus on creating comprehensive treatment strategies that incorporate microbiome profiling, genetic markers, and clinical characteristics of the disease.

This study significantly contributes to the understanding of the complex relationship between the gut microbiome and autoimmune arthritides, providing a foundation for innovative therapeutic approaches in rheumatology. The demonstrated clinical improvements following microbiome modulation highlight the promising prospects of this emerging field in personalized medicine.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: All studies were conducted in compliance with the ethical standards and principles of the Helsinki Declaration. The parents or legal representatives of all the study participants gave informed consent to participate in the study. The protocol of the experiment was approved by the local ethics committee (Protocol No. 45-12/2021)

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