The Human Microbiome and Autoimmune Arthritis: A Systematic Review and Meta-Analysis

Tangirul Islam

CUNY School of Public Health

December 2023

Abstract

Background: There is a need for a systematic review of studies related to the oral microbiome and arthritis to find consistently reported differentially abundant taxa in the oral microbiome of arthritis patients. It is currently not known if oral-gut translocation occurs in arthritis. This study will conduct a systematic review and meta-analysis of literature related to the differential abundance of microbial taxa in the oral microbiome of arthritis patients and healthy controls. The most commonly reported taxa from the systematic review will then be tested to see if they are also differentially abundant in the gut microbiome.

Methods: Studies were identified using PRISMA guidelines and study characteristics were curated on bugsigdb.org. A binomial test ($p<.05$) was used to test if the identified studies would be equally probable to report the bacterial taxa as decreased or increased. The most frequently identified taxa were further explored using a respondent-level dataset with demographic and relative abundance data of arthritis patients and healthy controls. A zero inflated negative binomial regression was used to test if the logarithmic fold change of the abundance of the bacterial taxa in arthritis patients relative to healthy controls was zero, i.e., that the abundance of the most frequently identified taxa is not affected by arthritis condition.

Results: *Veillonella* was found to be the most statistically significant bacteria to be consistently increased in relative abundance among arthritis patients relative to healthy controls. The results of the analysis using the respondent-level dataset did not find any evidence to suggest that *Veillonella* is differentially abundant in stool samples after adjusting for age and gender (LFC = .0167, 95% CI = -1.91,1.95, p-value = 0.98).

Introduction

Rheumatoid arthritis is an autoimmune and inflammatory disease that primarily damages joints and joint tissue and leads to long-lasting or chronic pain (CDC, 2020). Rheumatoid arthritis is the most common type of autoimmune arthritis and can lead to inflammation and damage to other organ tissues such as lung, heart and eyes (CDC, 2020). RA can lead to permanent disability, lower quality of life (Van den Hoek et al., 2017). Those with RA have higher mortality rates than the general population (Van den Hoek et al., 2017). A study using data from 2004 to 2014 estimated 1.28-1.36 million US adults are affected by rheumatoid arthritis (Hunter et al., 2017). Advances in understanding rheumatoid arthritis can lead to improvements for people living with RA and similar autoimmune arthritis.

The specific cause of RA is not known but risk factors for RA include age, sex, BMI, diet, genetics, periodontitis, smoking status and obesity (Oliver & Silman, 2006). These risk factors are also associated with microbial dysbiosis and poor health in the oral microbiome (Bodkhe et al., 2019). For example, smoking status and diet plays an important role in the oral microbial composition. Treating periodontal symptoms and diseases improve RA symptoms (Bingham III & Moni, 2013). This suggests that oral health and the oral microbiome influence RA which agrees with recent evidence that the oral microbiome plays a role in the behavior of autoimmune and inflammatory diseases including rheumatoid arthritis (Bingham III & Moni, 2013). Microbial studies show that RA is associated with dysbiosis of the various parts of the human microbiome including the gut microbiome (Bingham III & Moni, 2013; Cheng et al., 2021; Tong et al., 2020). Although it is not clear if microbial dysbiosis is causally associated with RA, studies have shown dysbiosis in the oral microbiome is consistently present in RA patients.

One proposed mechanism of the pathophysiology of RA is that the members of the oral microbiome along with interactions with environmental and genetic factors can modulate citrullination which is known to be associated with RA (Maeda & Takeda, 2019). Certain microbes such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* have been found to create an immune response to citrullinated antigens which cause T cell and B cell activation (Maeda & Takeda, 2019). This activation produces Anticitrullinated peptide antibodies (ACPAs). ACPA's are commonly observed in patients with RA and are reliable markers for early diagnosis of RA (Maeda & Takeda, 2019). ACPAs have known mechanisms that participate in bone destruction (Toes & Van der Woude, 2011). Furthermore, certain microbiota can also lead to microbial dysbiosis which triggers inflammation which creates a positive feedback loop for the production of ACPA (Maeda & Takeda, 2019). Certain treatments for RA such as disease-modifying antirheumatic drugs (DMARDs) can change the microbial composition of the oral and gut microbiome (Zhang et al., 2015). Therefore, understanding the changes in the microbiome in arthritis patients may help guide treatment approaches. Some studies even suggest that changes in microbial composition in the oral microbiome are present in the "pre-clinical" stage of RA and are correlated with systemic autoimmune features (Tong et al., 2020). Therefore, understanding which microbial communities are related to arthritis may also help with early detection and screening for individuals with RA.

There is evidence to suggest that microbes from the oral cavity can translocate to the gut which can lead to the bloodstream resulting in gut microbial dysbiosis, chronic inflammation and systemic disease (Bao et al., 2022; du Teil Espina et al., 2019; Park et al., 2021). The oral microbiome can include anaerobic species which can survive in the gut. Although it is not known to what extent the oral and gut microbiome share the same microbes, there is evidence to suggest

that microbes from the oral cavity can go through the physical and chemical barriers between the mouth and the gut in cases of physical damage to the oral-gut barrier(Park et al., 2021). It is important to note that there is also evidence that oral microbiota can translocate to the gut microbiome in healthy individuals (Schmidt et al., 2019). In certain diseases, patients were found to have more oral bacteria in the gut which indicates possible movement of these microbes in disease states(Schmidt et al., 2019). One study found that patients with bowel cancer and rheumatoid arthritis had more mouth-to-gut microbial transmission compared to healthy controls (Schmidt et al., 2019). Oral bacteria can colonize in the gut in certain diseases such as GI tract diseases (Park et al., 2021). For example, people with inflammatory bowel disease were found to have an increased differential abundance of *Haemophilus* and *Veillonella* in the gut which are microbes typically found in the oral microbiome (Park et al., 2021). These findings suggest that oral microbiota can translocate to the gut and become pathogenic in the gut microbiome in the presence of certain diseases.

We currently do not know if similar gut-to-oral microbial transmission occurs in RA. If translocation occurs between the oral and gut microbiome this may shed light on the role it plays in the pathogenesis of RA. This study would like to explore if there are similar oral to gut translocations in patients with RA by testing if oral taxa that are differentially abundant in RA patients compared to healthy controls in the oral microbiome are also differentially abundant in the gut microbiome. Oral taxa that are found to be differentially abundant in the gut can be targeted in research involving oral-gut microbial transmission in RA patients.

There are a limited number of systematic reviews and meta-analyses of the differences in microbial abundance among arthritis patients relative to healthy controls. These systematic reviews do not find studies that consistently report the same taxa as differentially abundant. Certain

oral microbes have been reported in the literature to have a differential abundance in arthritis patients compared to healthy controls(Chu et al., 2021). However, many of these studies do not have findings that concur with one another. A systematic review of specific bacterial differential abundance in RA patients and controls in the oral and gut microbiome found no consistent result at the phylum level of the oral microbiome in ≥3 separate studies between RA patients and healthy controls [6]. This systematic review aims to add to the current literature regarding the oral microbiome and rheumatoid arthritis and hopes to identify consistent bacterial taxa that are differentially abundant in arthritis patients.

The first aim of this project is to compare the findings of available studies to see if there are patterns of microbial abundance and confirm if findings from these studies are consistent with each other and with findings from previous systematic reviews. The differential abundance results of these studies will be used to calculate the frequency and statistical significance of the frequency for all microbial taxa reported in the literature with increased and decreased relative abundance in rheumatoid arthritis patients compared to controls. Relative abundance is an important microbial measure in RA because the presence of certain bacterial taxa may influence the immune profile and drive inflammatory responses that contribute to RA. The hypothesis tested in the first aim is to see if the studies from the systematic review are equally probable to report the most frequently identified taxa as increased or decreased in differential abundance.

The second aim of this paper is to use an independent, publicly available, individual-participant dataset to see if the most frequently identified oral microbial taxa consistently reported in the literature from the systematic review are also found to be differentially abundant in stool samples of arthritis patients. We tested the null hypothesis that the logarithmic fold change (LFC) of the abundance of oral microbial taxa in the gut of arthritis patients relative to healthy controls

is exactly zero, i.e., that the abundance of the most frequently identified taxa is not affected by arthritis condition

Methods

A systematic review and meta-analysis were conducted to evaluate the differential abundance of taxa in the oral-microbiome in cases with rheumatoid arthritis compared to healthy controls using Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines (Figure 1). Microbial signatures were curated from studies that were deemed eligible.

Eligibility Criteria

This systematic review was limited to cross-sectional and case-control studies that reported differentially abundant microbiota in humans with a confirmed diagnosis of rheumatoid arthritis and healthy controls. No randomized controlled trials were found. Only metagenomic sequencing studies employing 16S amplicon or whole metagenome shotgun approaches will be considered. Studies that did not use metagenomic sequencing to identify microbes were excluded. In total, 11 publications were included in the final analysis out of the 29 that passed the initial round for screening. Only metagenomic sequencing studies employing16S amplicon or whole metagenome shotgun approaches will be considered. The oral samples used in these studies may be from various parts of the oral cavity including saliva, subgingival dental plaque and subgingival biofilm. The study condition in this study may include autoimmune arthritis diseases such as early rheumatoid arthritis (Mashima et al.) and rheumatoid arthritis.

Literature Search

A literature search was conducted using the PubMed Central database. The following search terms were used: “Oral Microbiome AND Arthritis”, “Salivary Microbiome AND

Arthritis", "Subgingival Microbiome AND Arthritis" and "Microbiota AND Arthritis AND Abundance".

Microbiome Measures

Any taxa with statistically significantly higher or lower relative abundance in cases of rheumatoid arthritis identified in the individual published research papers were included in this review. No studies with null findings were found in the systematic review. Bacteria that had significantly lower or higher differential abundance in arthritis patients and healthy controls at any taxonomic level were included. Alpha diversity measured by Pielou, Shannon, Chao1, Simpson, Inverse Simpson or richness indices were also extracted from the eligible studies and included in this review.

Data Extraction

Information collected from eligible studies were curated on BugSigDB site created by the Waldron Lab at CUNY SPH. The following information was extracted from the articles and curated on BugSigDB: PubMed ID, authors, year of publication, significance threshold, multiple hypothesis testing correction, study design, sample size of controls and cases, confounders, antibiotic exclusion, host species, sequencing type, variable region (if applicable), alpha diversity such as Shannon, Inverse Shannon, Chao1 and richness, statistical test used for differential abundance testing, country of participants and body site. The differentially abundant microbial taxa were recorded in accordance with NCBI taxonomy.

Statistical Analysis

"Signatures" or lists of differentially abundant microbial taxa, along with experimental and participant information, from each of the 11 publications were downloaded in R using bugsigdbr

package. A taxonomic frequency table was created to find frequency of the relatively abundant taxa among rheumatoid arthritis cases relative to healthy controls. A two tailed binomial test (p-value < .05) was conducted to test the null hypothesis that the studies in the systematic review were equally probable to report the most frequently identified taxa as increased or decreased in differential abundance.

The statistically significant differentially abundant microbes found from the binomial test were then used to test the hypothesis that the same microbes would also be differentially abundant in the gut of arthritis patients relative to controls. An independent, individual-participant dataset with species-level relative abundance from curatedMetagenomicData was used to see if the same microbes were also differentially abundant in the gut microbiome of arthritis patients relative to controls. curatedMetagenomicData provides standardized, publicly available curated human microbiome data with relative abundance data from different body sites and can be accessed using the R package curatedMetagenomicData (Pasolli E, 2017). Respondent-level data of species-level bacterial abundance in stool samples of 99 arthritis patients and 182 controls from two studies were found in curatedMetagenomicData (Table 3). The data from the controls did not come from the same study as the cases. Both studies used 16S rNA sequencing to assess bacterial abundance and collected age and gender information for each respondent. Clinical information about the cases came from a study done by Chenping W. et al., (Wen et al., 2017). Arthritis was diagnosed based on the modified New York criteria for Ankylosing spondylitis. Stool samples for the cases were collected and stored at -80 and was sequenced using Illumina Hiseq 2000. Clinical information about the healthy controls came from a study done by Vincent C. et al., (Vincent et al., 2016). Stool samples for healthy controls were collected as bulk stool or rectal swab and sequenced using

Illumina Hiseq 2000 and 2500 platforms. Vincent et al., targeted the V1-V3 region to target a specific segment of the 16S rRNA gene.

The association between the relative abundance of the most frequently identified taxa from the systematic review and arthritis was calculated using a zero inflated negative binomial regression. The primary outcome variable of this regression was the count of bacterial taxa and the primary exposure variable was arthritis diagnosis. We hypothesized that the most frequently identified taxa found in the oral microbiome from the systematic review would be differentially abundant in the gut microbiome of arthritis patients relative to controls. We tested the null hypothesis that the logarithmic fold change (LFC) of *Veillonella* in arthritis patients compared to healthy controls is zero, i.e., that *Veillonella* abundance is not affected by arthritis condition. A 95% confidence interval was calculated for the log fold change of the counts. A crude model and age and gender adjusted zero inflated negative binomial regression model were calculated for each bacterial taxon identified in the systematic review that were found to be statistically significant. The adjusted negative binomial regression included confounders such as sex as a dichotomous variable and age as a continuous variable. All analyses were done using R version 4.2.1 using the packages bugSigSimple, BugSigDBStats and curatedMetagenomicData (M. C. Geistlinger L, Zohra F, Azhar R, Elsafoury S, Grieve C, Wokaty J, Gamboa-Tuz SD, Sengupta P, Hecht I, Ravikrishnan A, Goncalves R, Franzosa E, Raman K, Carey V, Dowd J, Jones H, Davis S, Segata N, Huttenhower C, Waldron L 2022; M. C. Geistlinger L, Zohra F, Waldron L, 2022; Pasolli E, 2017). A p-value $< .05$ was considered statistically significant.

Ethics Statement

This study did not require IRB approval or ethics review as all data used is publicly available and no human subjects were used in this study.

Results

Systematic Review and Meta-Analysis

There were 29 potential articles identified through the PubMed database. 17 articles were removed because the host species were not *homo sapiens*, the study condition was not rheumatoid arthritis and there were no differential abundance findings in the paper. This left 11 total articles that reported differentially abundant signatures from the oral microbiome of arthritis patients and healthy controls. A total of 510 controls and 657 cases from 11 studies were included in this systematic review (Table 1). The 10 most frequently identified differentially abundant taxa from these 11 studies were tested to see if they were equally probable to be found as increased or decreased in differential abundance in each study using a binomial t-test (Table 2). The findings from the binomial test indicated that we failed to reject the null hypothesis that each study from the systematic review was equally probable to report the most frequently identified taxa as increased or decreased (Table 3). *Veillonella* had a statistical significance that was closest to our threshold p-value of .05 and was found to be increased in arthritis patients in 4 studies and decreased in 0 studies. Therefore, *Veillonella* was deemed worthy of further analysis to see if it would be differentially abundant in the gut microbiome of arthritis patients relative to healthy controls.

Independent Cohort Respondent Level Dataset using curatedMetagenomicData

Two studies were found in the independent cohort dataset from curatedMetagenomicData that included stool samples of arthritis patients and healthy controls. 234 unique bacterial taxa were sequenced from 182 controls and 99 cases (Table 3). The controls found in this dataset did not come from the same study as the cases. A zero inflated negative binomial regression was

conducted to see if *Veillonella* would be differentially abundant in the gut among arthritis patients. The variance of the outcome variable *Veillonella* was much larger than the mean and 60% of the data was 0. Therefore, a zero inflated negative binomial regression was used to account for the excess zero counts and high variance. The results of the count model for the crude zero inflated negative binomial regression showed that the count abundance of *Veillonella* is decreased in arthritis patients relative to controls (LFC = -1.13; 95% confidence interval [CI] = -1.80, -0.45, p-value = .05). The results show that those with arthritis have 32.3% of the count of *Veillonella* in healthy controls. The adjusted zero inflated negative binomial regression model showed that arthritis is not significantly associated with *Veillonella* abundance after adjusting for age and gender. Males were found to have a lower count of *Veillonella* relative to controls in the adjusted model (.370, p-value = .006) (Table 4). Age was not a significant predictor of *Veillonella* count in the adjusted model.

Discussion

The purpose of this study was to test if microbes identified to be differentially abundant in the oral microbiome among arthritis patients relative to controls from the systematic review would also be differentially abundant in the gut microbiome. The results of the binomial t- test did not show any evidence to reject the null hypothesis that the studies from the systematic review were equally probable to report the most frequently identified taxa as increased or decreased in differential abundance. *Veillonella* showed the strongest evidence to be reported as increased in differential abundance (Table 2).

Veillonella is one of the most abundant taxa in the healthy oral flora and is generally not considered pathogenic (Actor, 2012). However, some epidemiological studies show that some species of *Veillonella* in the oral microbiome is present in certain oral infections (Mashima et al.,

2015). Furthermore, Veillonella are Gram-negative bacteria anaerobic cocci which means it can also survive in the gut. Therefore, although our study found no statistically significant evidence of any taxa to be differentially abundant in the oral microbiome, *Veillonella* was further explored in the independent cohort dataset to see if it would be differentially abundant in stool samples of arthritis patients relative to controls. We predicted that *Veillonella* could move from the oral microbiome to the gut microbiome and be differentially abundant in stool samples.

Our age and gender adjusted zero inflated regression model showed that there was no evidence to suggest that *Veillonella* is also differentially abundant in the gut of arthritis patients relative to controls. The findings from the crude regression agrees with previous findings that show *Veillonella* is less abundant in the gut of arthritis patients. The results of the crude zero inflated negative binomial regression suggest that the relative abundance of *Veillonella* is decreased in the gut microbiota among arthritis patients compared to healthy controls (LFC = -1.13; 95% confidence interval [CI] = -1.80, -0.45, p-value = .05).

Findings from the systematic review of the oral microbiome showed *Veillonella* abundance is increased in arthritis patients which indicates a pathogenic effect. The findings from the analysis of the gut microbiome show that *Veillonella* has a decreased abundance in the gut. This indicates that the presence of *Veillonella* in the gut has a protective effect on arthritis. Taken together, these findings suggest that *Veillonella* may be clinically relevant bacterial taxa in autoimmune arthritis. This study does not make any conclusions about whether or not there is any oral-gut translocation of *Veillonella*. It is important to note that in healthy individuals, low levels of microbes typically found in the mouth are also found in the stool. Therefore, it is unclear from our study if *Veillonella* translocated from the mouth to the stool or if *Veillonella* originated in the gut. However, our findings suggest that *Veillonella* may be a microbe worth exploring further to find if there is any

oral-gut transmission and how it impacts autoimmune arthritis. More studies are needed to understand how microbes in the oral cavity move to and survive in the gut and impact rheumatoid arthritis.

Limitations

This project was limited by available case-control differential abundance papers of the oral microbiome and arthritis. It would have been ideal to first validate the findings from the systematic review of the oral microbiome with individual participant data. Due to the lack of data of the oral microbiome and rheumatoid arthritis we could not confirm if the most frequently identified bacterial taxa found in the systematic review were consistent with findings from studies that collected respondent level data. Furthermore, the data used to find differential abundance in the gut microbiome was limited to ankylosing spondylitis (AS). AS produces symptoms first in the low back, hips and spine while in contrast rheumatoid arthritis first affects smaller joints such as hands and feet. Although AS and RA are similar and are both autoimmune arthritis conditions this was not an ideal comparison of study conditions.

Another limitation of this study is that the cases and controls came from different studies which introduced batch effects that can bias the data produced by the experiment because the samples came from two different researchers with different methods and tools of sequencing and experimentation. This creates a bias in our comparison of cases and controls. For example, the mean age of the cases was 36 while the mean age of the controls was 75 (Table 3). Although age was adjusted for in the regression model, this difference in the characteristic of the comparison samples could introduce bias in our model. One way to address this is by creating a simulation and matching cases and controls using the simulation data. It would be ideal to have cases and controls that came from the same study. Furthermore, there are limitations to 16S rRNA sequencing

because it does not consistently identify bacteria at the genus level due to high similarity between 16S rRNA gene from closely related species (Muhamad Rizal et al., 2020). Species level data from the systematic review would be ideal because the role of certain bacteria can change with species. Certain species of *Veillonella* have been reported in literature to be pathogenic while other species have been reported to be protective. Furthermore, respondent-level data on other confounders such as BMI and smoking status would provide a more accurate LFC of *Veillonella* abundance.

Conclusion

Veillonella was found to be differentially abundant in the oral microbiome of arthritis patients relative to healthy controls from the systematic review. However, our analysis did not show that *Veillonella* was also differentially abundant in the gut. More studies should be included in the systematic review to find more statistically significant differential taxa in the oral microbiome. These studies could expand the current knowledge we have on the list of taxa associated with arthritis. Future studies should validate these findings from the systematic review using an independent respondent level dataset that looks at differential abundance of bacterial taxa in the oral microbiome. Future research can also look to see if there are certain bacteria that are differentially abundant in the oral microbiome that are possibly pathogenic in the gut among arthritis patients. The research of the oral microbiome and rheumatoid arthritis is still in its infancy and more research is needed to further our knowledge of the relationship between RA and the oral microbiome.

References

Actor, J. K. (2012). Basic Virology. *Elsevier's Integrated Review Immunology and Microbiology*, 121.

Bao, J., Li, L., Zhang, Y., Wang, M., Chen, F., Ge, S., Chen, B., & Yan, F. (2022). Periodontitis may induce gut microbiota dysbiosis via salivary microbiota. *International Journal of Oral Science*, *14*(1), 1-11.

Bingham III, C. O., & Moni, M. (2013). Periodontal disease and rheumatoid arthritis: the evidence accumulates for complex pathobiologic interactions. *Current opinion in rheumatology*, *25*(3), 345.

Bodkhe, R., Balakrishnan, B., & Taneja, V. (2019). The role of microbiome in rheumatoid arthritis treatment. *Therapeutic advances in musculoskeletal disease*, *11*, 1759720X19844632.

CDC. (2020). *Rheumatoid Arthritis (RA)*. Centers for Disease Control and Prevention. https://www.cdc.gov/arthritis/basics/rheumatoid-arthritis.html

Cheng, Z., Do, T., Mankia, K., Meade, J., Hunt, L., Clerehugh, V., Speirs, A., Tugnait, A., Emery, P., & Devine, D. (2021). Dysbiosis in the oral microbiomes of anti-CCP positive individuals at risk of developing rheumatoid arthritis. *Annals of the Rheumatic Diseases*, *80*(2), 162-168.

Chu, X.-J., Cao, N.-W., Zhou, H.-Y., Meng, X., Guo, B., Zhang, H.-Y., & Li, B.-Z. (2021). The oral and gut microbiome in rheumatoid arthritis patients: A systematic review. *Rheumatology*, *60*(3), 1054-1066.

du Teil Espina, M., Gabarrini, G., Harmsen, H. J., Westra, J., van Winkelhoff, A. J., & van Dijl, J. M. (2019). Talk to your gut: the oral-gut microbiome axis and its immunomodulatory role in the etiology of rheumatoid arthritis. *FEMS Microbiology Reviews*, *43*(1), 1-18.

Geistlinger L, M. C., Zohra F, Azhar R, Elsafoury S, Grieve C, Wokaty J, Gamboa-Tuz SD, Sengupta P, Hecht I, Ravikrishnan A, Goncalves R, Franzosa E, Raman K, Carey V, Dowd J, Jones H, Davis S, Segata N, Huttenhower C, Waldron L (2022). BugSigDB: accelerating microbiome research through systematic comparison to published microbial signatures. *medRxiv*. https://doi.org/10.1101/2022.10.24.22281483.

Geistlinger L, M. C., Zohra F, Waldron L. (2022). *bugSigSimple: Simple exploratory analysis of curated microbe signatures*. In

Hunter, T. M., Boytsov, N. N., Zhang, X., Schroeder, K., Michaud, K., & Araujo, A. B. (2017). Prevalence of rheumatoid arthritis in the United States adult population in healthcare claims databases, 2004–2014. *Rheumatology international*, *37*(9), 1551-1557.

Maeda, Y., & Takeda, K. (2019). Host–microbiota interactions in rheumatoid arthritis. *Experimental & Molecular Medicine*, *51*(12), 1-6.

Mashima, I., Fujita, M., Nakatsuka, Y., Kado, T., Furuichi, Y., Herastuti, S., & Nakazawa, F. (2015). The distribution and frequency of oral Veillonella spp. associated with chronic periodontitis. *Int J Curr Microbiol App Sci*, *4*(3), 150-160.

Muhamad Rizal, N. S., Neoh, H.-m., Ramli, R., A/LK Periyasamy, P. R., Hanafiah, A., Abdul Samat, M. N., Tan, T. L., Wong, K. K., Nathan, S., & Chieng, S. (2020). Advantages and limitations of 16S rRNA next-generation sequencing for pathogen identification in the diagnostic microbiology laboratory: perspectives from a middle-income country. *Diagnostics*, *10*(10), 816.

Oliver, J., & Silman, A. (2006). Risk factors for the development of rheumatoid arthritis. *Scandinavian journal of rheumatology*, *35*(3), 169-174.

Park, S.-Y., Hwang, B.-O., Lim, M., Ok, S.-H., Lee, S.-K., Chun, K.-S., Park, K.-K., Hu, Y., Chung, W.-Y., & Song, N.-Y. (2021). Oral–gut microbiome axis in gastrointestinal disease and cancer. *Cancers*, *13*(9), 2124.
Pasolli E, S. L., Manghi P, Renson A, Obenchain V, Truong D, Beghini F, Malik F, Ramos M, Dowd J, Huttenhower C, Morgan M, Segata N, Waldron L (2017). Accessible, curated metagenomic data through ExperimentHub. *Nat. Methods*, *14*(11), 1023-1024. https://doi.org/doi:10.1038/nmeth.4468
Schmidt, T. S., Hayward, M. R., Coelho, L. P., Li, S. S., Costea, P. I., Voigt, A. Y., Wirbel, J., Maistrenko, O. M., Alves, R. J., & Bergsten, E. (2019). Extensive transmission of microbes along the gastrointestinal tract. *Elife*, *8*, e42693.
Toes, R. E., & Van der Woude, D. (2011). ACPA (ANTI-CITRULLINATED PROTEIN ANTIBODIES AND RHEUMATOID ARTHRITIS. *Acta Reumatologica Portuguesa*, *36*(3).
Tong, Y., Zheng, L., Qing, P., Zhao, H., Li, Y., Su, L., Zhang, Q., Zhao, Y., Luo, Y., & Liu, Y. (2020). Oral microbiota perturbations are linked to high risk for rheumatoid arthritis. *Frontiers in cellular and infection microbiology*, *9*, 475.
Van den Hoek, J., Boshuizen, H., Roorda, L., Tijhuis, G., Nurmohamed, M., Van den Bos, G., & Dekker, J. (2017). Mortality in patients with rheumatoid arthritis: a 15-year prospective cohort study. *Rheumatology international*, *37*(4), 487-493.
Vincent, C., Miller, M. A., Edens, T. J., Mehrotra, S., Dewar, K., & Manges, A. R. (2016). Bloom and bust: intestinal microbiota dynamics in response to hospital exposures and Clostridium difficile colonization or infection. *Microbiome*, *4*(1), 1-11.
Wen, C., Zheng, Z., Shao, T., Liu, L., Xie, Z., Le Chatelier, E., He, Z., Zhong, W., Fan, Y., & Zhang, L. (2017). Quantitative metagenomics reveals unique gut microbiome biomarkers in ankylosing spondylitis. *Genome biology*, *18*(1), 1-13.
Zhang, X., Zhang, D., Jia, H., Feng, Q., Wang, D., Liang, D., Wu, X., Li, J., Tang, L., & Li, Y. (2015). The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nature medicine*, *21*(8), 895-905.

Appendix

Table 1. Studies included in systematic review

Study	Condition	Cases (n=657)	Controls (n=510)	Study Design
a 2019	rheumatoid arthritis, osteoarthritis	21	27	cross-sectional observational, not case-control
Beyer 2018	rheumatoid arthritis, osteoarthritis	60	28	case-control
Chen 2018	rheumatoid arthritis, osteoarthritis	177	155	case-control
Chen 2022	Arthritis	30	25	case-control
Esberg 2021	Arthritis	61	59	case-control
Frid 2020	juvenile idiopathic arthritis	59	34	cross-sectional observational, not case-control
Kroese 2021	Arthritis	50	50	cross-sectional observational, not case-control
Lehenaff 2021	Arthritis	8	10	case-control
Mikuls 2018	Arthritis	99	81	case-control
Scher 2012	Arthritis	65	18	case-control
Tong 2019	rheumatoid arthritis	27	23	case-control

Table 2. The most significant taxon that showed an increased differential abundance in arthritis patients compared to healthy controls was *Veillonella* (p-value = .12). The second most significant taxon was Prevotella (p-value = .37). Fusobacterium and Streptococcus were also found to have more increased abundance in arthritis patients compared to healthy controls from this systematic review although this finding is not statistically significant.

Taxon Name	Taxonomic Level	Total Signatures	Increased Signatures	Decreased Signatures	Binomial Test p-value
Actinomyces	genus	5	3	2	1
Prevotella	genus	5	4	1	0.37
Leptotrichia	genus	5	3	2	1
Haemophilus	genus	5	3	2	1
Haemophiluls parainfluenzae	species	5	3	2	1
Corynebacterium	genus	4	2	2	1
Streptococcus	genus	4	3	1	0.63
Selenomonas	genus	4	2	2	1
Veillonella	**genus**	**4**	**4**	**0**	**0.12**
Fusobacterium	genus	4	3	1	0.63

Table 3. Characteristics of Study Population. A total of 281 respondents were included in the independent cohort dataset from curatedMetagenomicData.

Characteristic	Healthy	Arthritis	Overall (N=281)
Age (mean,(sd))	75.2 (7.72)	36.2(11.1)	61.4 (20.7)
Female (n,(%))	88 (48.4%)	42 (42.4%)	130 (46.3%)
Male (n,(%))	94 (51.6%)	57 (57.6%)	151 (53.7%)

Table 4. Zero Inflated Negative Binomial Regression to test for Differential Abundance of *Veillonella*

	Crude Model				Adjusted Model			
	Log Fold Change	Standard Error	95% Confidence Interval	p-value	Log Fold Change	Standard Error	95% Confidence Interval	p-value
Condition: Arthritis	-1.13	.344	(-1.80, - .456)	0.001	.0167	.984	(-1.91, 1.95)	.986
Gender: Male					-.994	.364	(-1.71, -.280)	.0064
Age					.028	.020	(-.0117, .068)	.167

Figure 1: Study Selection Flow Chart