The effectiveness of probiotics in the management of inflammatory arthritis

A thesis submitted in the fulfilment of the Master of Clinical Science

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Abstract

Objective**:** To systematically identify, appraise and synthesis evidence of the formulation specific effects and population specific responses of probiotics in inflammatory arthritis (IA).

Methods**:** MEDLINE (PubMed), CINAHL, EMBASE, and SCOPUS databases were searched for studies utilising probiotics and an intervention of inflammatory arthritis. The Joanna Briggs Institute (JBI) method was used to conduct the systematic review. A single reviewer undertook screening and data extraction. Two independent reviewers assessed the quality of evidence using JBI tools.

Results**:** A total of 154 full text articles were retrieved and of these twelve eligible studies were reviewed. Of these, ten (83%) were randomised controlled trials and two (17%) were quasi-experimental studies. Four studies included a variety of spondyloarthopathies (SpA). Eight studies focused on rheumatoid arthritis (RA). Probiotics were supplied for a median timeframe of 60 and mode of 56 days (range 7-365 days). Overall, 17 different probiotics were supplied in colony forming units (CFU) per 24hrs ranging from 1x 10^8 to 2.25 x 10^{11}. The genus of probiotic most commonly supplied was Lactobacillus.

There was no statistical difference in the relative risk (RR) of minor adverse effects between probiotic and control groups (RR 1.02, 95% CI 0.69 to 1.51) when including nil event studies and no major adverse effects reported. However, effects were more often reported for studies on SpA. Meta-analysis identified a statistically significant benefit of probiotics on quality of life with a standard mean difference (SMD) effect size -0.37 (CI-0.59, -0.15), p=0.01 with subgroup analysis favouring Lactobacillus-only formulations. Negative effects sizes related to the reduction in quality of life scores that utilise a higher score to indicate worsening symptoms and more impact upon daily living. Small but statistically significant

reductions in pain, p=0.006 with a mean difference effects size -8.97 (95%CI -15.38, -2.56), were identified independent of formulation. Meta-analysis confirmed the known statistically significant benefit of probiotics on the inflammatory marker C-reactive protein p=0.017 with a mean difference effects size -2.34 (95%CI -4.26, -0.41), with subgroup analysis demonstrating a greater difference in RA and combined Bifidobacterium and Lactobacillus formulations. The clinical significance of these small changes is questioned.

Conclusion: This review indicates a potential differential benefit to combined formulations of Bifidobacterium and Lactobacillus compared to purely Lactobacillus formulations, with respect to reducing pain, lowering C-reactive protein and improving quality of life. It also suggests altered benefits dependant on the type of inflammatory arthritis with less benefit and more frequently reported side effects for individuals with SpA compared to RA. Generalisability of results to clinical practice is limited by the dominant demographic of older individuals, with established disease beyond the 'therapeutic window of intervention' for Inflammatory arthritis. Small but statistically significant benefits require confirmation using clinical studies with greater consideration to confounding factors of age, gender, diet and individual microbial signature.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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CHAPTER ONE Introduction

1.1 Structure of the thesis

This thesis is structured in four chapters. The first chapter provides a broad introduction to the topic under review, in order that the following methodology and results sections can be understood in context.

The introduction begins by explaining the common forms of inflammatory arthritis and the need for exploring adjuvant interventions to current care, despite the advances in pharmacotherapy in the past ten years. This chapter then goes on to provide a background to the development of modern-day probiotics and explores the current understanding of their mechanisms of action, with the resulting benefits and safety issues. To appreciate the range of probiotic actions and their allocation to specific strains, the introduction also contains a section on the phylogeny and classification of probiotics. An explanation of common outcome measures applied in rheumatology is provided to rationalise the outcomes selected for the systematic review. Finally, the introduction concludes by examining the current systematic reviews that have been undertaken in the area. This serves to justify the construction of this systematic review and the gaps in the literature it aims to address.

The second chapter outlines the methodology of the review including the population, intervention, comparators and outcomes of the studies to be included as well as the justification and explanation of studies excluded from the review.
The third chapter provides the results of the systematic review process and includes the PRISMA flowchart, details of the quality appraisal of all included studies, forest plots for comparison of effect sizes, where appropriate, and a narrative summary of the results of the included studies.

The fourth and final chapter provides the discussion of the overarching thesis topic. It starts with a discussion of the evidence of the efficacy of probiotics according to outcomes of the systematic review and progresses to discuss the evidence relevant to specific forms of inflammatory arthritis or specific formulations of probiotics. The impact of study quality, population characteristics and the probiotics strains supplied are discussed along with

sources of bias and reliability in outcome measurement. The thesis is concluded with consideration of the limitations of the review, suggested areas for future research and recommendations for practice.

A brief overarching conclusion is provided prior to the references and appendices.

1.2 Inflammatory arthritis

Chronic inflammatory arthritic diseases have a multifactorial aetiology characterised by auto-antibody production and systemic features. Synovial inflammation induces pannus and joint destruction unless aggressively managed early by disease modifying therapies. Inflammatory arthritis can be highly disabling with immense personal, social and economic costs. Healthcare costs alone for rheumatoid arthritis (RA) in Australia were estimated at $550 million in 2015.[1(p.3)] Worldwide, the prevalence of RA has been estimated at 0.24% and responsible for 4.8 million disability-adjusted life years (95% CI 3.7 million to 6.1 million) in 2010.[2] With the number of people with arthritis estimated to rise to 5.4 million people by 2030 this correspondingly creates a higher burden of disease and costs to individuals and governments.[3] The family of spondyloarthropathies (SpA) including psoriatic arthritis, ankylosing spondylitis, reactive arthritis, inflammatory bowel disease associated arthritis (also known as enteropathic arthritis) and undifferentiated SpA have considerable variation in reported prevalence estimates.[4]However, recent estimates of disease impact suggest the burden from SpA is similar to that experienced by those living with RA.[5] Disease burden can be estimating in many ways, increasingly patient reported outcomes (PROMs) are viewed as effective measures of the impact and burden of disease, as serum measures of systemic inflammation such as C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) do not correlate strongly to other markers of disease activity or functional or quality of life outcomes.[6]

1.3 Aetiology of inflammatory arthritis and the role of dysbiosis

Whilst debate continues over the aetiology of inflammatory arthritis, a multifactorial aetiology is generally accepted. There is emerging evidence that microbial dysbiosis at mucosal sites (in concert with environmental triggers) can be involved in the disease in genetically predisposed individuals.[7] Key findings supporting this theory include that elevated serum-related auto-antibodies have been found in early RA without clinically

evident synovitis, suggesting that pathology develops outside of the joint.[8] The gut has been suggested as one key mucosal site which may trigger auto immune reactions in distant sites such as the joints. This hypothesis stems from identification of dysbiotic gut microbiota in individuals with early stage auto-immune inflammatory RA, which can be partly normalised after treatment.[8,9] Such is the specificity of the dysbiotic changes that genetic markers of these gut microbes can be used to identify individuals with RA from a control group.[10] Microbial dysbiosis has been most widely researched in people with RA, but there is growing evidence to link microbial dysbiosis with the whole clinical spectrum of inflammatory arthropathies. Studies are now emerging on the gastrointestinal microbiota in people with SpA and juvenile idiopathic arthritis. Evidence exists which supports an even closer link between gut microbiota and the pathogenesis of SpA than observed in RA.

Subclinical gut inflammation has been estimated to occur in up to 70% of patients with ankylosing spondylitis and up to 100% of patients with psoriatic arthritis.[11,12] This association may have been masked by a lack of reportable bowel symptoms despite the prevalence of colonoscopic changes in patients with psoriatic arthritis and psoriasis.[12] Similarly, subclinical evidence of gut inflammation has been found on magnetic resonance enterography of a sample population of juvenile arthritis patients. Whilst the small sample size in this study may increase the likelihood of type II error, the prevalence of clinically diagnosed inflammatory bowel disease is also higher for individuals with juvenile arthritis than in the general population.[13,14] Whilst gut changes have been identified in RA there remains debate between researchers as to whether intestinal inflammation is a primary abnormality or occurring as a result of the effects of medications, such as non-steroidal anti-inflammatories.[15]

1.4 Current management options and limitations

Pharmaceutical management using disease modifying anti-rheumatic drugs (DMARDS) remains the mainstay of intervention for inflammatory arthritis, often coupled with non-pharmacologic management strategies. However, adverse drug effects are commonly experienced, and remission is not guaranteed. About 20% to 40% of patients treated with newer version biological disease modifying anti-rheumatic medications– the tumour necrosis factor inhibitors - fail to achieve a 20% improvement in American College of

Rheumatology criteria and more lose response over time (secondary failure or acquired therapeutic resistance).[16,17] In juvenile arthritis, new pharmaceutical therapy has increased the likelihood of attaining inactive disease in up to 70-90% of children within two years, yet sustaining remission remains problematic for almost 50% of all children.[18] Similarly in ankylosing spondylitis only a certain percentage of patients achieve partial remission which may prompt the search for alternative management options.[19] New studies indicate that, with the application of the new targeted biological medications, there may be much higher rates of sustained clinical remission, potentially up to 50% of patients with ankylosing spondylitis may now achieve remission, highlighting a defined window of opportunity for effective intervention.[20] However, this trial excluded patients with later disease (defined as more than 12 weeks after diagnosis) and those with more extensive disease (defined as poly-articular disease, five swollen joints or more).[18]

Even for those who can access biologics, who can respond and sustain that response over time – there are patients who are unwilling to accept ongoing pharmacotherapeutic interventions or look to supplement them with alternative therapies.[21] As earlier diagnosis and intervention becomes the norm, there is also a possibility that a greater number of individuals will perceive early provision of disease modifying anti-rheumatic medications as an overly aggressive and unwarranted management strategy and look for alternative and adjuvant approaches given the lesser magnitude of their symptoms. A recent systematic review into the patient perceived health service needs in inflammatory arthritis identified that there are many different drivers that may encourage the high rate of complementary and alternative medicine use.[22] The review identified patients' perceptions of an ongoing need for symptom management, a desire for more holistic consultations and shared decision making, alongside financial disincentives and negative past experiences of traditional pharmaceutical management, may all play pivotal roles in the use of complementary therapies in inflammatory arthritis care.[22]

1.5 Complementary and supplementary therapy usage

The use of supplementary and complementary therapies by patients as a means to seek, or maintain, symptom control remains high, and a search for adjuvant interventions to reduce the burden of disease in inflammatory arthritis remains common.[22] As specific data of

probiotic usage in rheumatology clients is lacking, this review aims to generate capacity for clinicians to understand the likelihood of benefits and harms from the application of current probiotic formulations and be confident in discussing these. Such discussions form part of a holistic approach desired by patients and that are perceived by patients to improve communication and their feeling of autonomy in the ongoing management of their condition.[22]

Despite a lack of rigorous trials, probiotics are a booming business. The global probiotics market totalled $US 34 billion in 2015 and is set to grow to $US50 billion by 2020.[23] Whilst levels of probiotic supplementation in rheumatology populations in Australia are unknown, research has provided evidence that up to a third of the community does use probiotics with little concern about side effects and without informing health professionals about their use.[24,25] If probiotics are considered within the wider category of complementary and alternative medicine, and a significant number of people with inflammatory arthritis are using them, this highlights the perceived inadequacies or unacceptability of standard therapies for these conditions.[26,27]

A survey from Australia indicated that 94.7% of a sample of 75 ankylosing spondylitis patients reported previous or current complementary therapy use.[28] Clinicians may not appreciate the widespread use as research indicates that almost half are not reporting such use to their health care team. Whilst a full understanding of the rationale for such non-disclosure is not currently known, studies by Rao and Robinson would indicate that the past experience, or anticipation of, a negative response from clinicians is part of the reason that complementary therapy use is hidden in formal healthcare interactions.[29-31]

The reasons for complementary therapy use have been explored, and include a perception of lower risks/harms, alongside the value of being in control of their treatment choices.[32] The use of complementary therapies in ankylosing spondylitis has been identified as more likely in women with higher levels of education, suggesting that use may be interpreted as an indicator of greater desire of autonomy, consistent with management principles for long-term health conditions.[28] Harnessing patients' desire for involvement in their healthcare by understanding the evidence base for, and engaging in discussions about complementary

therapies, seems a more effective means of shared-decision making than the current situation of disguised use and non-disclosure. This thesis aims to help enable open discussion of the role of probiotics as an exemplar complementary therapy in rheumatology.

1.6 Probiotics

Probiotics have been defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". [33(p.507, Box.1)] Probiotics are one of several potential 'therapies' that are being investigated regarding positive benefits for the gastrointestinal system. The GI system is home to most human microbiota, which confer significant benefit upon human hosts including contributing to an effective immune system that can tolerate safe commensal bacteria, whilst ensuring a rapid inflammatory response to pathogenic organisms.[34,35] A bourgeoning literature base has investigated the effects of probiotics on the gastrointestinal system, theorizing that they address 'dysbiosis' or imbalance in the gut microbiome, and can down regulate the pro-inflammatory cytokine cycle implicated in triggering auto immune diseases, such as inflammatory arthritis.[36-41]

The clinical benefits of probiotics on the microbiome have been most extensively studied in inflammatory bowel disease, where a systematic review and meta-analysis demonstrated that probiotics can induce remission and help prevent relapse.[41] Furthermore, in animal studies probiotics were capable of down regulating the pro inflammatory cytokine cycle.[42] The interest in harnessing probiotics proven anti-inflammatory responses for managing inflammatory arthritis is growing with the emerging evidence highly relevant to researchers, clinicians, industry and patients.[43]

However, alongside potential benefits there have also been potential risks identified.[43] Potential adverse health effects from probiotic use, such as systemic infection, deleterious metabolic activities, immune dysregulation and gene transfer, have been identified.[44] A recent systematic review revealed the most common adverse effects were noted in immune-compromised patients and included sepsis, fungemia and GI ischemia. Dangers should not be underestimated, as demonstrated where a multispecies probiotic was used enterally for individuals with acute pancreatitis. Mortality in the group which received the probiotics was 16%, significantly above that of the placebo group at 6%.[45] Therefore, rheumatologists, general practitioners and pharmacists should be well informed and

capable of discussing the evidence-based risk benefit ratio of probiotics with their patients.[46]Therefore, a rigorous identification, appraisal and synthesis of the current evidence is urgently needed to inform practice and enable reliable, accurate information for health consumers.

1.6.1 Historical context of probiotics

The term probiotic is derived from the Latin preposition 'pro' which means 'for' and the Greek word 'biotic' meaning 'bios' or 'life'.[47] Its earliest documented scientific use was in the early 1950's to describe a range of supplements that had restorative properties for human health. The term was more tightly defined by an article in *Science* which attributed the benefits specifically to the work of bacteria.[48,49] By 1974, it was suggested that a wide range of organisms beyond just bacteria could act as probiotics.[47] The current definition of probiotics was formulated in 2001 by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organisations (WHO) as "live microorganisms which, when administered in an adequate amount, confers a health benefit to the host".[33(p.507)]

However, probiotics are not really such a recent phenomenon. Most of the world's oldest cultures, including those of Asia, Africa and Australia, have practised forms of food fermentation using microbial cultures since 7,000 or more years BC, and these early fermented foods are the direct link to our modern probiotic products.[48] Gogineni [49] outlines how the understanding of probiotic mechanisms by the great French chemist Pasteur in 1860 was followed by the work of Henry Tissier, the first person to clinically apply lactic acid probiotics (LAB) by giving isolated Bifidobacterium to infants with diarrhea. It is now known that LAB create an acidic local environment, which inhibits the growth of harmful bacteria, preventing food spoilage and protects humans from pathogenic bacteria in the gut.[50]

Development of clinically validated commercial products has been more problematic as bacteria commonly used as yogurt starters (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were found to be incapable of colonizing the human intestine.[51]
Whilst Dr Minoru Shirota is acknowledged as the first to develop a commercial probiotic product capable of surviving the gastrointestinal tract, the impact of the great depression,

the world wars and development of antibiotics, slowed much further research into probiotics until the 1980's.[50] The rise of antibiotic resistance alongside the capacity of modern microbiology techniques to identify mechanisms of actions in the human body beyond lactic acid fermentation, has led to a resurgence in probiotic research (Figure 1).[52]

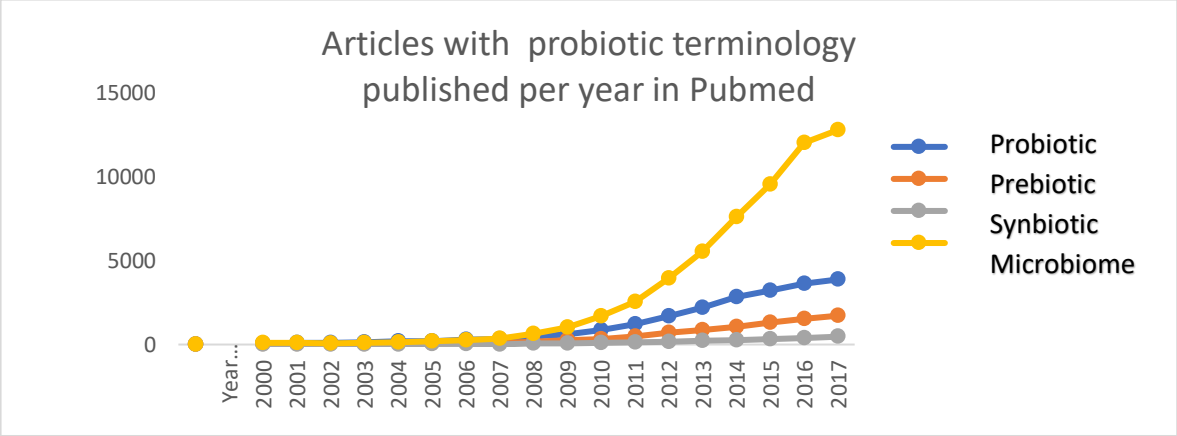


Figure 1. Rapid growth in research in the microbiota and probiotic arena, as displayed by publications identified with key words in Pubmed

Developments in health technology, such as microbiology molecular techniques, access to efficient large-scale genome sequencing and computer aided bioinformatics have revolutionised our understanding of the microbiota of the human GI system. Similarly, they have shed light on the far-reaching ways in which probiotics and their metabolites can impact human health, as will be discussed in greater detail in section 1.2.3. Through applying metagenomic analysis, the Human Microbiome Project has identified >40 000 species in the colon, however work to understand their complex actions, exquisitely tuned interactions and therapeutic potential has only just begun.[53-55]

1.6.2 Taxonomy and classification of probiotics

A clear understanding of classification becomes relevant when seeking to understand the mechanisms of action for probiotic formulations. Probiotics may include a variety of micro-organisms living in and on the human body that confers a health benefit including eukaryotes, archaea, bacteria and viruses. However, the majority of organisms that have been researched, applied and evaluated are bacteria or yeast.[55] There are still very few studies addressing non-bacterial components of the microbiome, including the virome,

despite increasing evidence of the link between reduced bacterial diversity and bacterial function with expansion of specific viruses in immune mediated disease, specifically Crohn's disease.[56,57]

This thesis will therefore focus on those organisms most commonly applied as probiotics currently: bacteria and yeast. Each organism will be referred to by its current brand name and nomenclature according to recognised taxonomic rules. It should be recognised, however, that there are significant challenges to the correct identification of probiotic strains, including a lack of a global standard. Some basic terms and concepts in bacterial taxonomy to understand throughout the thesis, are that 'Classification' is the process of clustering organisms into taxonomic groups (taxa) based on similarities or relationships. 'Nomenclature' is the assignment of names to the taxonomic groups according to international rules.

As genetic knowledge expands, classification can change resulting in the re naming of species. Whilst early classification was restricted to a limited number of organisms that could be cultured and grown *ex vivo*, the rise in genetic sequencing of *in vivo* samples has created complexity by generating several hundred thousand new species every year.[58] The proliferation of new species alongside increasingly precise genomic tools has created a shifting in taxonomy. Using genetic profiling to establish such relationships has created new phylogenetic trees that display the genetic (rather than morphological) relationships of organisms. (see Figure 2 below).

Figure 2 is adapted from the interactive tree of life and displays the hypothesised phylogenetic tree and placing of the common probiotic genera.[59-61] The four main genus of probiotics used currently are marked and named as *Saccharomyces, Escheria, Lactobacillus* and *Bifidobacterium*

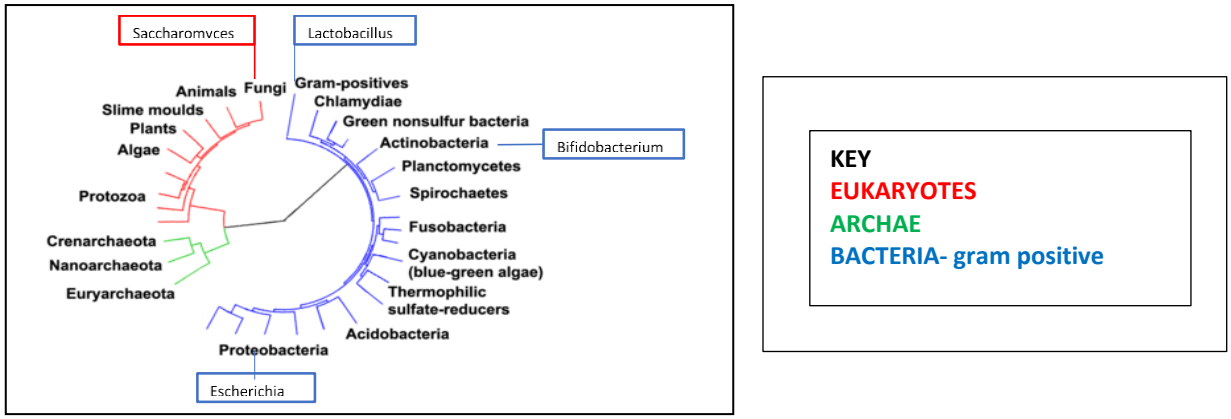


Figure 2. A highly resolved Tree Of Life, based on completely sequenced genomes [1]. The image was generated using iTOL: Interactive Tree Of Life[2], an online phylogenetic tree viewer and Tree Of Life resource.[65]

Such phylogenetic schemes may require alignment with traditional classification schemes, requiring a combination of profiling and identification methods. Where there have been changes in the naming and classification of probiotic species contained within studies, this has been flagged and discussed where required.The current three domain system groups organisms primarily based on differences in ribosomal RNA (rRNA) structure. Basically, all forms of life are classified into three domains and six kingdoms.

. A clear understanding of classification becomes relevant when seeking to understand the mechanisms of action for probiotic formulations, to clarify species applied in trials or commercial products, and identify risks of negative side effects. As shown in Figure 3 most probiotic organisms belong to the gram-positive type bacteria within the domain of Bacteria, the kingdom of prokaryotic organisms and the phylum Firmicutes. Within the classification of Firmicutes, the two most common Genus applied as probiotics are the Lactobacillus and Bifidobacterium genera[44] This is clarified within Figure 3 and it should be noted that throughout this thesis the terminology Bifidobacterium will be used to denote any probiotics that fall within the Bifidobacterium genus, and the term Lactobacillus will be used for those within the lactobacillus genus.

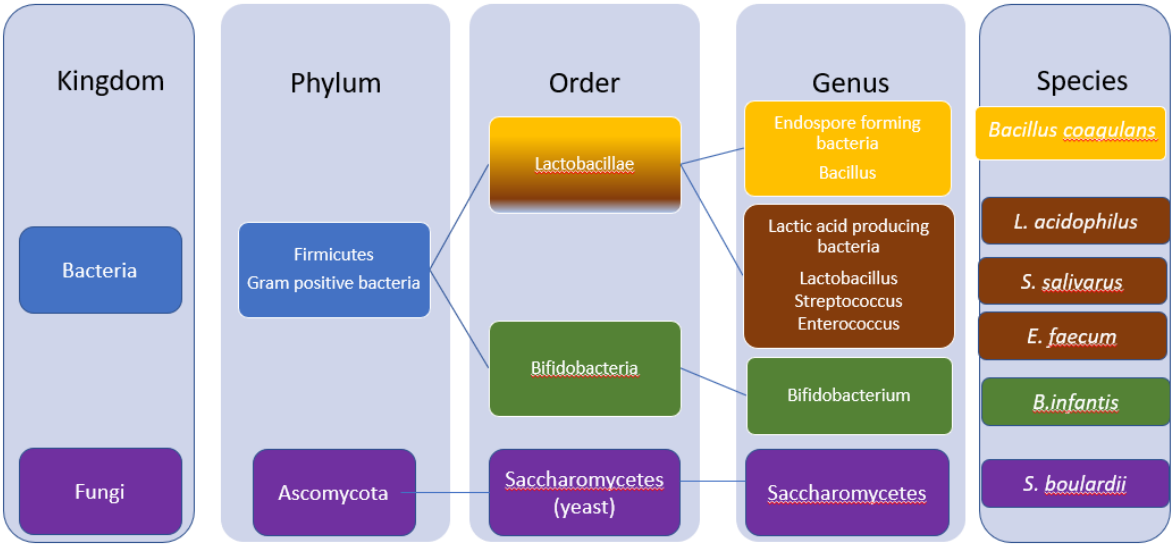


Figure 3 Simplified classification diagram to represent basic taxonomy of common probiotics

Classification at a species, or subspecies, level relies on 16S rRNA gene sequence technology. It has been determined that bacteria strains can be identified as belonging to the same species if they share 70% or more DNA relatedness (at the DNA–DNA hybridization or re-association level) and possess more than 97% 16S rRNA gene sequence identity.[59] The genus Lactobacillus is the most extensively studied with the most validated species, but Bifidobacterium species are also well characterised.[62,63]

Yeasts which have been applied as probiotic organisms are found within the kingdom of Eukaryotes. Yeasts are single-celled microorganisms classified as members of the fungus kingdom. They are a diverse group, and currently only organisms within the phylum of true yeasts, Ascomycota has been explored for commercial use as probiotics.[64]

1.6.3 Mechanisms of action

It has been a long-standing and commonly accepted paradigm that probiotics exhibit strain-specific effects.[64] This viewpoint would render a systematic review on different strains redundant, as treatment effects would not be able to be directly compared. However, advances in molecular technology are now revealing more complex and interwoven mechanisms of action, with shared actions within and across taxonomic groups for probiotics.[64] Proposed mechanism of action relevant to the two main groups of probiotics applied in the studies within this review (yeast and bacteria) is discussed below.

Yeast

The study of yeasts in dysbiosis is less developed than that of commensal bacteria, and primarily only the *Saccharomyces cerevisiae* has been studied in detail.[65] A range of key mechanisms for the antimicrobial actions of yeast have been suggested as nutrient competition, pH change caused by their production of organic acids or ethanol and secretion of antibacterial and anti-microbial compounds.[66]

For decades, researchers have known that the production of secondary metabolites by yeast, known as killer toxins or 'mycocins' were a cause of the antimicrobial properties of foods and drinks fermented by yeast.[67] Production of antibacterial compounds have been described across a range of yeast species, and are capable of inhibiting potentially pathogenic bacteria, for example, *Candida intermedia* can reduce *Listeria monocytogenes*.[68] They can also potentially alter the human commensal bacteria community, for example inhibition of *Lactobacillus plantarum* by yeasts has been reported.[69] In addition many placebo controlled clinical trials have demonstrated an effect of *Saccharomyces boulardii* against antibiotic associated diarrhoea and *Clostridium difficile* associated diarrhoea.[70] Research has indicated that mechanisms of action are likely to be multifactorial, including releasing bacteriostatic or bactericidal substances, which inhibit pathogenic effects of bacterial toxins, have anti-secretory action, and show trophic, immune-stimulatory and anti-inflammatory responses.[67] Investigation into the application of yeast probiotics for inflammatory bowel disease has hypothesised that that anti-inflammatory benefits are gained by the yeast altering the migration of T cells, reducing levels of pro-inflammatory cytokines and therefore gut inflammation.[71]

Bacteria

It is important for the translational science of probiotics that common benefits via known mechanisms can be ascribed, since insufficient studies have been undertaken on identical strains to permit systematic reviews that adhere to a strain specific mechanism viewpoint. Whilst it appears that a comprehensive understanding of all mechanisms for all species and strains is not yet elucidated, there are some well-evidenced examples of modes of action that can be provided, specifically for the most studied genera of Lactobacillus and Bifidobacterium.[69] Widely studied and well documented probiotic effects are discussed as

per Hill et al 2014[33] with more recent evidence supplied to substantiate specific mechanisms as relevant to this thesis. Probiotics have been shown to act in the gastrointestinal tract in different ways, which can be simplified and represented in four key areas (Figure 3).

Each effect can be created through several mechanisms and an example of common shared mechanisms across genera, and more strain specific mechanisms is provided. This aims to provide a brief rationale for the capacity to undertake a systematic review in probiotics and a rationale for the sub-population analysis which occurs by genera due to the number of different mechanisms of action shown between the Bifidobacterium and Lactobacillus genera. A simplified diagram of shared and common mechanisms is shown in Figure 4.

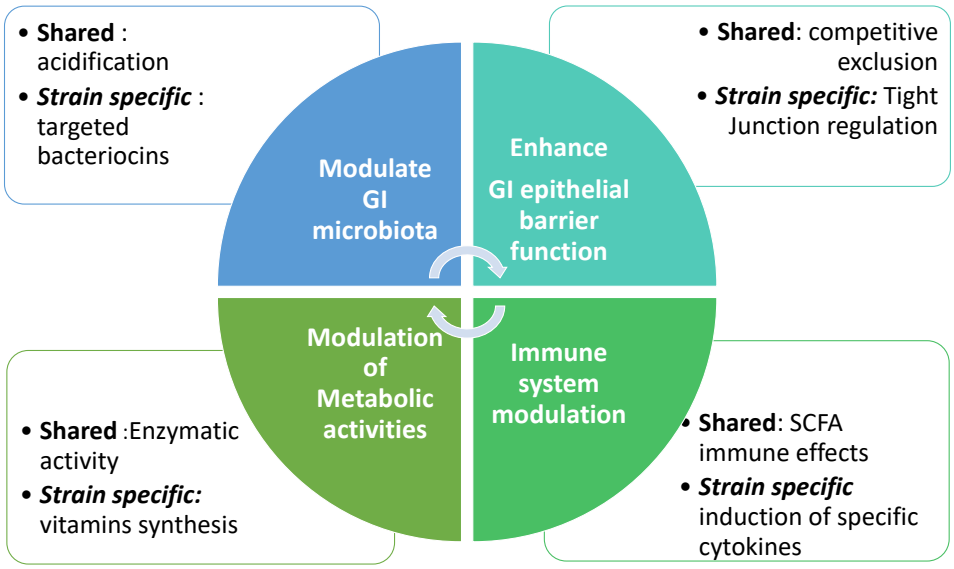


Figure 4 Diagrammatic representation of key probiotic mechanism of action with examples provided of shared and strain specific mechanisms (SCFA, short chain fatty acids)

Modulating the Gut microbiota: Lactic acid producing bacteria have been shown to demonstrate general anti-microbial resistance. This occurs through a common mechanism shared across many in the genus, since the production of lactic acid reduces gut pH locally, and is sufficient to reduce viability of potential pathogens.[72,73] Targeted bacteriocins may also be secreted by specific probiotic strains which have a focused and narrow action, providing the probiotic with a competitive advantage in the gastrointestinal tract for example Bifidocin B, which is produced by *Bifidobacterium bifidum NCFB 1454*.[74]

Enhancement of gut epithelial barrier function by probiotics has been demonstrated in many ways. Inflammation of the gastrointestinal tract, as in Inflammatory bowel disease, has been linked to infiltration of pathogens/microbes, due to loss of barrier function. The epithelial integrity is mainly controlled by tight junctions, which are protein complexes found at the apex of the epithelial cell. Most gram positive bacteria such as Lactobacillus and Bifidobacterium genera share cell surface molecules that enable them to adhere to the gastrointestinal epithelia, enhancing barrier function by facilitating competitive exclusion of other microbiota.[75] Certain very strain-specific mechanisms have also been discovered, for example, *Lactobacillus plantarum WCFS1* activates the Toll like receptors on the epithelial cells which up regulate the tight junctions, and therefore can enhance intestinal integrity.[69]

Modulation of the immune system by probiotics supplied to the gastrointestinal tract has been demonstrated through *in vivo* and *in vitro* studies and is discussed extensively in Dwivedi et al. 2016.[76] Immunodulation may be unsurprising considering approximately 70% of our body's immune system is located in the gut.[77,78] Many different probiotic taxa share the ability to produce short chain fatty acids which directly affect levels of monocyte/macrophage and neutrophil recruitment and the production of anti-inflammatory cytokines. All Bifidobacteria share the same metabolic pathway to create short chain fatty acids (known as the 'bifido shunt') and a recent comparative genomic analysis of publicly available Bifidobacterium genomes reveals that all enzymes within the shunt pathway are found across all species of Bifidobacterium.[64] Therefore, this anti-inflammatory action working through the immune cytokines may be considered phylum generic. Specific short chain fatty acids have also been shown to enhance the release of specific anti-inflammatory cytokines, for example butyrate enhances the release of IL-10, and there are differences in the ability of strains of *Lactobacillus plantarum* to induce IL-10 related to butyrate production.[69]

Modulation of metabolic activities is complex and can occur across many aspects of metabolic function. For example, the capacity for Lactobacillus species to produce enzymes (lactase or beta galactosidase) that can degrade lactose in the gut. This capacity is so well proven that the specific health claim that probiotics may 'improve the digestion of lactose' is currently the only claim recognised in the European regulatory market.[71] Another

potential probiotic metabolic activity is the hydrolysation of bile salts, which act to reduce the toxicity of bile to the probiotics cell membranes and therefore increases their intestinal survival and persistence. Bile salt hydrolase activity has been shown across the entire range of gram-positive bacteria.[79] Therefore, such metabolic functions may be considered shared mechanisms across a broad range of probiotics.

Probiotics also enable humans to obtain certain essential vitamins that they are unable to synthesis themselves. Vitamin synthesis capacity seems to be more strain specific, for example creation of folate requires two precursors, and genomic analysis of Bifidobacterium species suggests that only a few have the genes to code for both these precursors and could create folate in the gut of their host.[80,81] As a mainstay of rheumatology continues to include the use of Methotrexate, a known folate antagonist, patients currently require folate supplementation to offset the adverse effects of folate depletion.[82] The potential capacity of probiotics to create folate on demand in situ is an intriguing possibility for adjuvant therapy.

Given the complexity and overlap between actions and mechanisms, in addition to the deficit in complete genome typing across all strains and species, this review will attempt to provide some broad background to the formula of probiotics employed in the included studies, and where possible, draw links between demonstrated effects in human populations and known mechanisms of probiotic action.

1.6.4 Safety consideration of probiotics

With increasing knowledge of the mechanisms of action of probiotics there has also been more clarity as to the potential side effects that may occur.[43] Side effects have been suggested to occur for several reasons including, but not limited to :

A. Translocation /transmigration of probiotics across the gut barrier resulting in invasive infection i.e. bacteraemia or endocarditis;
B. Facilitation of transfer across gut membranes of pathogenic bacteria/species
C. Toxic or metabolic effects on the gut, i.e. ischaemia
D. Alteration of immune system function in a deleterious manner,
E. Transfer of antibiotic resistance between gut flora

A systematic review into the safety and side effects of probiotics published in 2013 identified that opportunistic infection from ingested probiotics in immune compromised and hospitalised patients has rarely been observed, and led to clinical cases of sepsis, fungaemia and gut ischemia.[44] As the majority of patients living with inflammatory arthritis are taking a range of immune supressing medications from traditional steroids through to biological disease modifying anti-rheumatic medications this indicates a higher degree of caution should be exercised when evaluating a risk: benefit ratio for probiotic use in this population.

Safety profiles were found to vary across probiotics and study authors identified that most bacteraemia cases were associated with the delivery of *Lactobacillus rhamnoses* and the most fungaemia cases associated with *Saccharomyces boulardii*.[44] The majority of evidence at this time suggests that serious adverse effects remain rare (based on epidemiologic data) and that there is little increase risks based on usage. However, there is a lack of trials that have aimed to investigate adverse events directly or establish any important dose dependant relationships that may exist. The risk of human infectious disease due to Lactobacillus is considered to be less than one case per million individuals.[83] There are two accepted safety status labels applied to probiotics in the food industry: Qualified Presumption of Safety (QPS) by the European Food Safety Authority (EFSA) and Generally Recognised as Safe (GRAS) by the United States-Food and Drug Administration (US-FDA).[84]

The potential for translocation depends partially on the adhesive qualities of the bacteria for the gut epithelium. However greater adhesive qualities, do not necessary equate to greater risk of harm as this preferential adhesion can also exclude other pathogenic organisms.[83] Direct toxic effects on the gut, due to metabolites produced by bacteria, can also occur. Such metabolite toxicity was identified in the PROPATRIA trial and resulted in significant increases in mortality in the group of advanced pancreatitis patients receiving probiotics.[45] There is no universal system currently in place to categorise the safety of microorganisms such as probiotics, however a four-level Risk Group system is commonly used to categorise the risk that any microorganisms may pose to humans. For example, see the World Health Organization system, summarised in figure 5.[85]

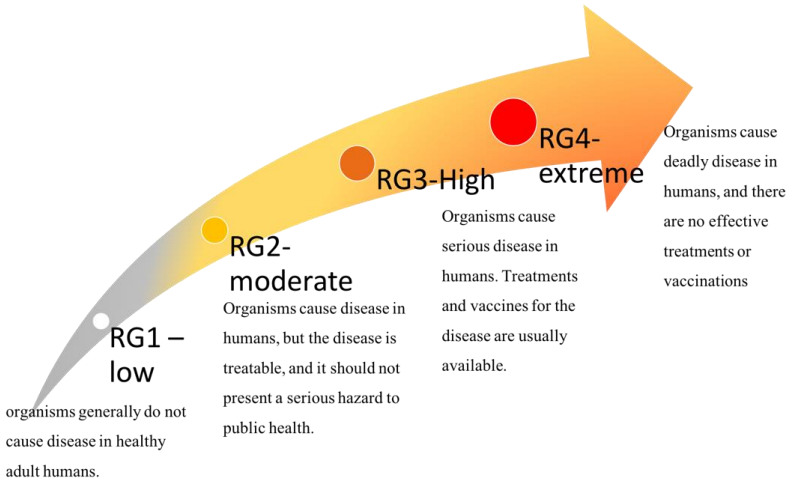


Figure 5. Modified diagrammatic representation of World Health Organization four-level Risk Group system adapted from WHO laboratory safety manual [85]

A cautious approach to probiotic administration remains advisable, as specific strains may be exceptions to the rule in relation to risk assigned to their phylum. For example, whilst most lactic acid bacteria may be considered safe, the enterococcus genus includes strains that can also be opportunistic pathogens and which can lead to bacteraemia and infections.[86] Therefore, active surveillance for adverse effects should be a core activity whenever clinical trials employ probiotics.[87]

Known strains of probiotic organisms have been shown to harbor resistance to antibiotics. There is a natural evolutionary occurrence of antibacterial resistance within certain bacterial species, which has long been part of their survival mechanisms. However, there is also the capacity for specific antibiotic resistance to be transferred between bacteria allowing bacteria pathogenic to humans to develop new forms of antibacterial resistance.[87]As antibiotic resistance continues to present a major global health concern, this side effect whilst currently theoretical, requires close consideration. A wide range of antibiotic resistance genes have been found in the gut biota of healthy populations across the world, creating potential for administered probiotics to facilitate the passage of these antibiotic resistance genes to pathogenic bacteria through horizontal gene transfer.[88] Such horizontal gene transfer is more likely when bacteria contain mobile genetic elements such as plasmids and transpoons, which are present in many lactic acid bacteria. [88]

Whilst surveys on rheumatology patients views on the risks of probiotics are lacking a review of patients living with Inflammatory bowel disease concluded that "Patients viewed probiotics as an appealing alternative to pharmaceutical drugs and understood probiotics as a more natural, low-risk therapeutic option".[89 (p. 138)] This view should be evidence based and therefore this review will aim to extract data relating specifically to safety and side effects in the rheumatology population.

1.7 Measuring outcomes in rheumatology

In general, medicine and therapeutic interventions aim to make a significant difference to the lives of patients in a manner that is meaningful to them. Historically, patient-centred outcomes have not necessarily predominated, as they are often not as easy to measure as biological markers. The international consortium known as OMERACT (Outcome Measures in Rheumatoid Arthritis Clinical Trials) has worked to place priorities of patients at the forefront of rheumatology research. They have done this by embedding patients as stakeholders and using evidence-based assessments of measurement tools in the development of a core set of outcomes.[90]

This thesis has included and categorised outcomes in a modified OMERACT manner, as appropriate to probiotics research as shown in Figure 6 below. OMERACT identifies four core areas, death, pathophysiological manifestations, life impact, and resource utilisation.[94] The domain of pathophysiological mechanisms captures disease activity and therefore includes systemic inflammation, both at the joints and within the body as a whole. Separate domains have been developed for individual inflammatory forms of arthritis , for the basis of this thesis the RA OMERACT guidelines provided a starting point for identifying outcomes relevant to both the condition and the intervention of probiotics.[91]

For the purposes of this thesis, all aspects related to adverse events whether considered minor reported outcomes, such as gastrointestinal upset or flatulence, or major reported outcomes, such as sepsis, interaction with concurrent standard care/medication or mortality, will be considered under the core domain of adverse events rather than simply mortality /death.

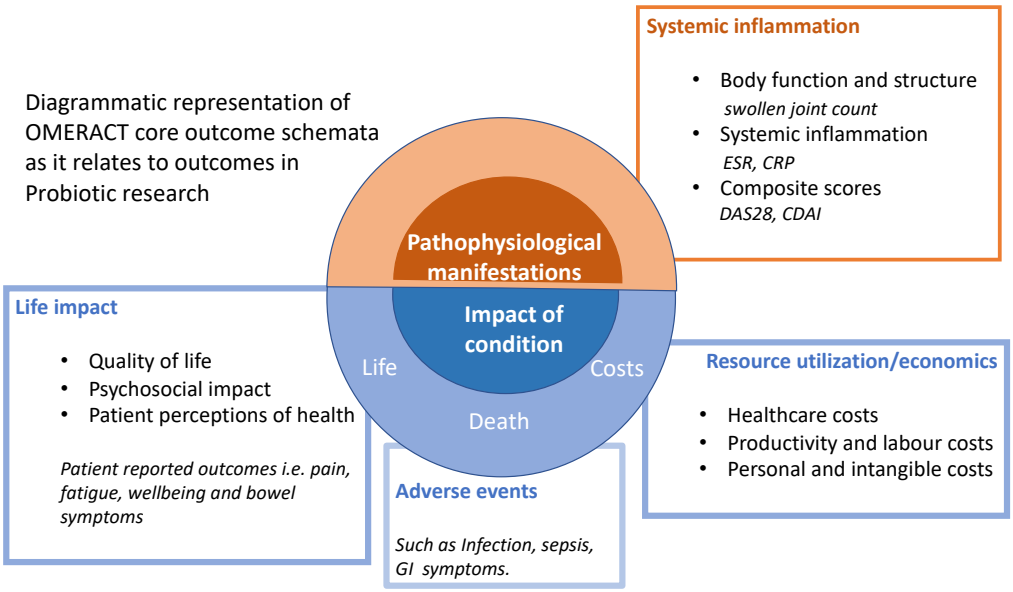


Figure 6 Modified representation of OMERACT core measures and domains as they relate to probiotic research.

1.7.1 Adverse events

Adverse events may be defined in variable ways, but for the purpose of this thesis they are described as an unexpected medical problem that happens during treatment with a drug or other therapy. Adverse events may be mild, moderate, or severe, and may be caused by something other than the drug or therapy being given.[92] Occurrence of an adverse event does not necessarily attribute causality or mean that the medicine/intervention was the cause of the event. Adverse events may include side effects, which are the known unintended effects of a medicine or treatment, adverse drug reactions, or drug interactions. There is the potential that reported adverse effects may be additional symptoms of the underlying concurrent condition for which the medicine is being taken.

Improving reporting in rheumatology trials has been part of the OMERACT focus as acquisition of adverse event data in clinical trials has been described as 'highly variable' and that formal assessment of safety and tolerability lags far behind that for efficacy.[93]

In this thesis, the Common Terminology Criteria for Adverse Events (CTCAE) as shown in Table 1 will be utilised as a way of grading the reported events reported in primary studies, where appropriate.[92]

Table 1 Common Terminology Criteria for Adverse Events (CTCAE) Version 4.02 [96]

Classification	Symptom severity	Modification of treatment
Grade 1	Asymptomatic or mild	Observation only
Grade 2	Moderate	Minimal non-invasive intervention or limitations of ADL
Grade 3	Severe	Requires hospitalisation and disabling
Grade 4	Life threatening	Urgent intervention required
Grade 5	Death	

A key outcome measure when research affects the gut microbiota is identifying effects upon gut function, which may be related to change in microbial community, change in metabolic function, gut integrity or epithelial function. Adverse effects on the gastrointestinal system will be considered separately to bowel outcomes, although there is clearly overlap and debate as to whether change in bowel function would be considered an adverse effect, adverse reaction, expected or unexpected.

1.7.2 Systemic Inflammation

When considering 'what matters' to patients, aspects that relate to pathophysiological mechanisms such as markers of systemic inflammation, are often rated poorly.[94] The outcomes desired from a patient perspective include functional improvement and the ability to live well with their condition experiencing meaningful and productive lives. However, with a plethora of new medicines being used to intervene and achieve early disease control or 'remission' – there remains a need for relatively quick, inexpensive and easy-to-interpret ways for health practitioners to monitor disease activity and response to intervention. Acute phase reactants, such as the erythrocyte sedimentation rate and C reactive protein, are commonly used as a measure of inflammation in rheumatology, therefore they are specified within the core set of disease measures for RA by the American College of Rheumatology and European Union League Against Rheumatism.[95] Acute phase reactants may be considered descriptive biomarkers, as they reflect disease state but are not specific to any given rheumatic condition and may be raised in other inflammatory and /or infectious conditions.[95] They are discussed below.

1.7.3. Erythrocyte Sedimentation Rate (ESR)

ESR is a surrogate marker of acute inflammation. Refer to Table 2 for details.

Table 2 Characteristics of the outcome measure Erythrocyte Sedimentation Rate.

OMERACT core area	Action	Specificity	Affected by	Relationship to other markers
Systemic Inflammation ESR	Reflects acute-phase plasma proteins in the blood, i.e. fibrinogen.	Non-Specific so likelihood of false positives.[96] Slow response to the acute phase reaction so likelihood of early false negatives	Age, gender and body mass index (BMI).[97]	Predictor of swollen joint count ($p < 0.001$) and correlated to CRP.[98]

*CRP (C reactive Protein) ESR (erythrocyte sedimentation rate)

As a simple validated disease assessment measure its use may be appropriate in probiotic trials as it may reflect inflammation in the gastrointestinal system and joints, as long as careful interpretation of the impact of the trials population demographics (age, gender and body mass index) on baseline values also occurs.[97]

1.7.4 C-Reactive Protein (CRP)

C-reactive protein is an acute phase reactive protein directly involved in early inflammation. Refer to Table 3 for details.

Table 3. Characteristics of the outcome measure C-Reactive Protein

OMERACT core area	Marker details	Specificity	Affected by	Relationship to other markers
Systemic Inflammation CRP	Measure specific acute phase inflammatory proteins so levels fall rapidly once the inflammatory cascade stops.[96]	Still non- specific to the disease-causing inflammation so likelihood of false positive remains.[96]	Gender and body mass index (BMI) [97]	Direct comparison showed ESR and CRP are significantly correlated with each other, swollen joint counts, and common composite measures.[99]

*CRP (C reactive Protein) ESR (erythrocyte sedimentation rate)

Many studies tend to favour C-reactive protein over erythrocyte sedimentation rate when assessing RA inflammation, because of its more rapid response time. As change in gut microbiome and inflammatory bowel function may be occurring as quickly as 3 days after dietary change this makes it a more suitable marker for probiotic studies.[100]

1.7.5 Immunological markers

Elevation of specific cytokines has been identified in RA and other immune modulated musculoskeletal diseases, leading to the development of medications that block these specific cytokines. Such cytokine blocking therapies have revolutionised the management of rheumatic conditions. Cytokine levels and expression are being explored as outcome

measures in rheumatology.[101,102] Similarly to probiotics, a true understanding of their complex systems, which contain considerable synergy and redundancy, currently limit the interpretation of cytokines used as biomarkers in clinical research for all areas including that of probiotics.[102] Therefore, studies that only utilise biomarkers as outcomes were not included. Brief details of their utility and application are provided in Table 4.

Table 4 Characteristics of cytokines as an outcome measure

OMERACT core area	Marker details	Specificity	Affected by	Relationship to other markers
Systemic Inflammation **BIOMARKERS I.e. Cytokines**	Small proteins, which play important roles in cell signalling and regulation of the immune response.	Can be predictive for an individual's response to therapeutic interventions, as the marker may be directly involved in disease pathogenesis.[101]	Age and gender [103,104]	Investigations suggest cytokines correlate with patient's function , serum markers of inflammation and composite measures in RA and AS. New [105,106]

*AS (Ankylosing spondylitis) RA (rheumatoid arthritis)

1.7.6 Composite measures

Given the complexities of chronic inflammatory forms of arthritis and the impact they can have upon an individual's life, composite outcome measures or disease indices, that incorporate core concepts from OMERACT alongside biomarkers have been developed which aim to provide coverage of a wider number of outcome domains. As such measures cut across core components and domains, and individual data contributing to subjective and objective elements of the score cannot routinely be disaggregated, composite scores will be analysed and discussed separately. Whether individual or composite outcome measures are utilised, there remains the challenge of identifying recognizable endpoints and determining whether statistically significant changes in scores are clinically important, and meaningful to the patient.[96]

Validated minimal clinical differences that result in meaningful change for patients and identify response to treatment for each outcome measure will be discussed in greater detail in the results section. A range of commonly applied disease indices are discussed in Table 5. Joint count assessment are components of all the indices above, they are undertaken by the clinician and are widely used in clinical trials, research, and day-to-day practice. It is a practical low cost traditional method of identifying clinical synovitis, that is still used despite the development of more advanced imaging techniques such as magnetic resonance

imaging.[107] It remains an important monitoring component, as ongoing joint swelling in patients in remission is known to correlate to radiographic progression. Joint counts may be considered as a semi-objective clinical measure.

Table 5 Characteristics of common disease indices in Rheumatology

core area **Systemic Inflammation**		Disease activity score (DAS)	Simplified Disease activity index (SDAI)	Clinical disease activity Index (CDAI)
Components	Joint count	Swollen count Richie articular index for tender joint	Swollen count Tender count	Swollen count Tender count
	marker	ESR or CRP	CRP	None
	Patient scale	General health scale (0-10)	Physician Global assessment of disease activity Patient global assessment of disease activity	Physician Global assessment of disease activity Patient global assessment of disease activity
Scoring		Based on an equation that incorporates weighted values of the Richie Articular Index and scores for other elements.	The individual components are not weighted but a simple sum	Ranges from 0 to 600, a score of 150 is defined as the threshold between remission and active disease
Reliability		Composite reliability 0.85 and 0.86 for the DAS28-ESR and DAS28-CRP, respectively [110,111]	0.88[112]	0.89[110]
Test retest		When looking at individual elements the swollen jointcount has been found to have the lowest reliability.[110] Test–retest reliability of patient-reported measures was satisfactory .[111]		
Internal Consistency Cronbach's alpha		Whilst reliability has been demonstrated – may view Cronbach's Alpha as an inappropriate internal consistency measure with an index measure.[110]		
Criterion validity		DAS28 correlates well to SADAI and CDAI. [117]	correlated well with the ACR response criteria HAQ, and DAS28 (r > 0.8).[116]	Correlated to both DAS28 and HAQ [117]
Development		Developed for Inflammatory Arthritis from ACR response criteria,	Derived from the Disease Activity Index for Reactive Arthritis	Derived from the SDAI
versions		The modified DAS28 compares favourably with the 44-joint version in early RA.[113]	One version only	One version only
Limitations/comments		DAS may be performed using an ESR or CRP as a serum marker. DAS28-CRP and DAS28-ESR are interchangeable according to recent research.[113]	Do not follow a normal distribution, discrepancy between the SDAI and DAS28 in patients with low levels of disease activity[113]	More stringent measure compared to the DAS28 when classifying patients in remission or with a minimal residual disease activity. [121]

*AS (Ankylosing spondylitis) CRP (C reactive Protein) ESR (erythrocyte sedimentation rate) RA (rheumatoid arthritis) ACR (American College Rheumatology)

A recent systematic review identified that whilst intra-observer reliability was good, inter observer reliability was poor especially in the swollen joint count.[108] This difference was

suggested to occur because of a higher dependency of the swollen joint assessment on factors like the assessors' levels of training and experience, a lack of standardization in examination methods, unclear definitions of swelling, or the degree of joint deformity. [109] Whether the administration of probiotics can meaningfully affect the swelling in synovial joints within the timeframe of most clinical trials is unknown, however as key rheumatology outcomes embedded within current practice they have been included for this thesis.

1.7.7 Life impact

Patients' perspective on the impact of their conditions, and its medical management, has become a recommended aspect of outcome assessment in clinical trials. Not only is including patient-driven outcomes desirable but research has suggested they can be as effective as traditional composite outcomes.[114] This efficacy extends to both identifying short term change in disease activity and predicting long term outcomes.

There are a wide range of health-related quality of life instruments to measure life impact of rheumatology conditions, although this doesn't guarantee that these tools prioritise aspects specifically rated as important by patients. Evidence suggests there remains disparity between clinician and patient viewpoints.[115]

Relevant outcomes listed within the core OMERACT domain of life impact includes: pain, morning stiffness, fatigue, sleep disturbance, patient's global rating of function and/or wellbeing, physical functioning as described through activities of daily living such as the Health Assessment Questionnaire (HAQ), mental health and wellbeing (for example anxiety, depression) and health-related quality of life. Life impact outcomes likely included within the studies of this review are further discussed below.

1.7.8 Pain

Definition

The International Association for the Study of Pain defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage.[116] Pain is not just a physical sensation. It is influenced by attitudes, beliefs, personality and social factors. Pain can affect emotional and mental wellbeing. Traditionally, acute pain is seen as a short-

term experience (lasting less than three months) which relates only to the timeframe within which tissues are healing and nociceptive signals are being triggered by tissue damage. After this time, an ongoing experience of pain has been termed chronic or persistent pain, as it lasts beyond the time expected for healing, has become an independent entity and is characterised by changes within the central nervous system.

Pain in Inflammatory arthritis

In rheumatological conditions, a simple paradigm existed for many years with all joint pain being determined nociceptive pain, due to the stimulation of nerve endings by joint inflammatory processes. This is partly true, for at the outset of an inflammatory condition acute pain will be driven by inflammatory molecules within joints. These inflammatory messengers fire signals along the somatosensory neuron, via the dorsal horn, to the spinal cord and finally to the brain, where the pain is registered in our consciousness. The inflammatory cascade releases further chemicals locally which lower the firing threshold for nerves, so that normal movement and sensation can also be interpreted as painful. In a situation of chronic and intermittent inflammation, as in many rheumatic diseases, pain becomes more complex.[117] Ongoing sensitisation of the peripheral and central nervous system can ensue, creating joints continually sensitive to normal ranges and pressures despite the absence of inflammatory chemicals. Descending pathways from the central nervous system can facilitate the maintenance of pain states, and neuroplasticity can ensue, both responding to and creating the pain experience of any given individual.

Mechanisms for alteration of pain by probiotics

There is some evidence that probiotics may have the capacity to influence pain via a variety of mechanisms. A systematic review concluded that the weight of evidence from 77 studies indicates that probiotics are able to "modulate the immune system, down regulate the inflammatory factors of immune system, reduce proliferation of T-Cells, and reduce proinflammatory cytokines".[118 (p.6)] Murine studies support this showing the capacity of supplementation with the probiotic *Lactobacillus rhamnosus* to increase the acute pain threshold in mice, the authors indicating that preferential stimulation of anti-inflammatory cytokines, such as IL2, may be the mechanisms by which the pain sensitivity was changed.[119] Alternatively, a broader explanation has been explored looking at communication between

the central nervous system and the enteric nervous system, termed the gut- brain axis. It has long been known that there are neural (via the vagus nerve and autonomic nervous system) and hormonal (via adrenocorticotrophic hormone and cortisol) methods of communication that enable the brain to influence intestinal function and immune cells.[120] New knowledge suggests a more bi-directional communication along the gut-brain axis, which may be modulated by the resident microbiota or applied probiotics. Studies have confirmed that microbiota may affect anxiety via brain neurochemistry and that a change in microbiota associated with the supply of probiotics has been able to reduce visceral hypersensitivity.[121,122]

Measuring Pain

Given the new insights into the pain picture in rheumatic diseases, it has been recognised that managing pain requires a multimodal approach and that innovative pharmacological approaches should be considered.[123] Simple direct patients' reports of pain using a visual analogue scale (VAS) are commonly used in rheumatology and there are a number of important elements to consider when looking at a change in VAS. Firstly, it is important to identify the minimal clinically important difference or MCID. MCID has been defined as the "smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate…a change in the patient's management".[124 (p.408)] Defining a MCID for any given scale requires a reference standard or anchor against which to benchmark the scale, in rheumatology the patient global assessment (PGA) is often used as the relevant anchor. Where improvement is the required outcome, the term minimal clinically important improvement (MCII) may be used as a specific reference.[125]

VAS may be represented in different orientations and with different end of scale annotations. It has been assessed to be a valid measure of pain intensity in rheumatology patients.[126,127] Whilst some cut-off points have been recommended: no pain (0–4 mm), mild pain(5-44 mm), moderate pain (45–74 mm), and severe pain (75–100 mm), they have been formulated in acute pain post-surgical environments that may differ from the complex pain experience of rheumatology patients.[128] The approach taken to combine statistical and meaningful clinical difference, with standardised wording, for this thesis will follow that suggested by the American Academy of Orthopaedic Surgeons, (Table 6).[128]

Table 6 Level of efficacy used in Osteoarthritis treatment guidelines depending on the statistical and minimal clinically important improvement (MCII) of the treatment effect From American Association Orthopaedic Surgeons 2013[128]

Descriptive term	Condition of use	Rationale
Clinically significant	Statistically significant	lower limit of CI > MCII
Possibly clinically significant	Statistically significant	CI contains the MCII
Not clinically significant	Statistically significant	upper limit of CI < MCII
True negative finding	Not statistically significant	upper limit of CI < MCII
Inconclusive finding	Not statistically significant	CI contains the MCII

*CI (Confidence interval) MCII (minimal clinically important improvement)

1.7.9 Fatigue

Fatigue is defined by the dictionary as "a state of extreme tiredness, typically resulting from mental or physical exertion or illness".[129] It is a common symptom and a recent online survey identified it as a prevalent problem affecting 50% of adult patients across 30 different rheumatic diseases.[130] Rates of fatigue were found to vary across conditions with prevalence reported as 41% in RA, 45% in ankylosing spondylitis and highest at 57% in psoriatic arthritis.[130] A clear consensus definition of fatigue in rheumatology is currently lacking, however Aaronson expands the definition to "a subjective, unpleasant symptom which incorporates total body feelings, ranging from tiredness to extreme exhaustion, creating an unrelenting overall condition which interferes with an individual's ability to function to their normal capacity".[131 (p.527)] As fatigue is common across the rheumatic conditions and has major consequences on patients' lives, there is now international consensus that fatigue should be evaluated in clinical trials for inflammatory arthritis.[132-134] Table 7 provides detail on measures used in this review.

Whilst there are many hypotheses about the stimulation and sustenance of fatigue in auto immune inflammatory conditions, the causes of fatigue are currently considered multifactorial, and have not been proven to be correlated with severity of disease state or systemic inflammation.[135,142-144] There are many instruments that assess fatigue that have been used in research, and an in depth review of all available fatigue measures is provided by Hewlett et al.[145] There is no gold standard fatigue instrument for rheumatologic conditions and some of the commonly used measures are discussed briefly below in Table 7. Investigating the potential effects of probiotics on the specific symptom of fatigue is important as it is already known that even in those patients who respond well and achieve

remission with DMARDS, only approximately a third achieve resolution of their fatigue symptoms.[136]

Table 7 Characteristics of two common fatigue indices used in Rheumatology

OMERACT core area **Life Impact- Fatigue**		Multi-dimensional assessment of fatigue (MAF)	Visual analogue fatigue scale (VAS Fatigue)
		16 item scale with four dimensions: degree and severity, distress that it causes, timing of fatigue), and its impact on of daily living.[135]	unidimensional scales and can be used for scoring the discrete components of severity, duration, or intensity
Admin		Self-administered. Higher scores indicate more fatigue.	
Scoring		Total Score from zero (no fatigue) to 50 (severe fatigue).	May be 0-10 or on a 100 mmm scale.
Reliability	Test retest	Not found	coefficient of 0.70 in RA (23) [136]
	Internal Consistency	Cronbachs alpha 0.93 [137]	Cronbach's alpha 0.91– 0.96 [136]
Sensitivity		Sensitivity to change was demonstrated	VAS fatigue in RA is more strongly associated with clinical variables indicating it is more "sensitive to change performs as well as or better than longer. [135 (p.1896)]
Pop validity		Developed for adults with RA	Taken out of Bath Ankylosing spondylitis disease activity index fatigue assessment
Normative data [141]	Healthy	17.0 (±11.3)	No healthy norms established , failure to differentiate fatigue and sleepiness [138]
	RA	29.2 (± 9.9) in RA	Median fatigue severity s 45 mm [138]
	AS	32 (SD 20) in AS.[135]	Fatigue commonly observed (67% population n=639) with average 42.1 SD 11.9 in those with fatigue.[139]
	PsA	Data not found	Mean 3.79 (SD 3.09) [140]
Limitations/comments		Easy-to-use with a low patient burden, good reliability and validity. However can underestimation of fatigue and its impact upon life.[141]	VAS, MAF, and SF-36 vitality subscale in a large RA cohort scales correlated well with each other (r 0.71–0.8) and with clinical measures (r 0.5–0.63).[141]

*AS (Ankylosing spondylitis) RA (rheumatoid arthritis) VAS (visual analogue scale) SF-26 (Short form 36)

1.7.10 Bowel function

A key outcome measure when researching gut microbiota is identifying effects upon gut function, which may be related to changing microbial community, change in metabolic function, gut integrity or epithelial function.[146] If, as hypothesised, the SpA share disease mechanisms with the inflammatory bowel disorders, then gut inflammation and changes in tolerance to commensal bacteria are a primary sign of all these diseases and may be expected to alter in response to the delivery of probiotics.[146] It has also been suggested that

bowel symptoms should always be involved in monitoring disease activity in the SpA as clinically relevant bowel symptoms may be overlooked amongst.[147] Tools designed specifically for rheumatology patients are not yet in use, therefore a brief review of common outcome measures currently used in trials is provided in Table 8.

Table 8 Characteristics of three common patient reported outcomes for bowel symptoms

OMERACT core area **Adverse effects- GI**		Dudley Inflammatory Bowel Symptom Questionnaire (DISQ)	Visual analogue scale for bowel symptoms (VAS IBS)	Gastrointestinal symptoms rating scale (GSRS)
Admin		self-report measure, consisting of 15 questions	Self-report measure on 7 items- rated 0- 10	interview-based rating scale of 15 items
Scoring		Original scored on a five-point numerical rating scale (0 = none/never to 4 = incapacitating). The score of the DISQ ranges from 0 -60. Higher scores indicate greater severity	abdominal pain, diarrhoea, constipation, bloating/ flatulence, vomiting/ nausea, perception of mental well-being, symptoms' effect on daily life.[148]	Items scored from 1 to 7. then dividing by 15 to obtain the final GSRS score between 1 - 7. The higher the overall score, the more severe the symptoms.
MCID		MCID that identifies bowel symptoms enough to affect QoL is 11 (out of a maximum of 60).	FDA recommending that ≥30% decrease of VAS IBS be considered a clinically significant endpoint. [160]	MCID of the GSRS total score was assumed to be 0.33 score points.[149]
reliability	Test retest	coefficient correlation for Spa 0.57[147]	coefficient correlation ranges from 0.4 to 0.8 for different items [154]	coefficient correlation ranges from 0.36–0.75.[150]
	Internal	0.79[147]	0.85 [148]	0.43–0.87
Development		for bowel symptoms of IBD	for use in IBS	for use in IBS
Pop norms		controls 2.6 (2.6), SpA 8.7 (6.1)[132]	Clarifies symptoms rather than create a final score. Stats sig difference between controls and patients with IBS.[148]	Normative scores have been set as less than 2.[149]
Criteria validity		Reliable measure for SpA.[133] Strong correlation shown with the CDAI (r = 0.98)[147]	Correlation has been shown between the VAS IBS and the DISQ .[148]	
versions		The SpA modification of the DISQ consists of 15 questions scored similarly	Caution as studies may only use certain components	A paediatric version suitable for children with JIA (the GSRSK)[151]
Limitations/comments		Rheumatology Population have only been used to develop reliability, validity and population norms for the DISQ, therefore transferability to other rheumatology patients remains uncertain.		

*AS (Ankylosing spondylitis) CDAI(Clinical disease activity index) IBS(Irritable bowels syndrome) MCID(Minimal clinically important difference) QoL(Quality of life) RA (rheumatoid arthritis) SF-26 (Short form 36) SpA (Spondyloarthritis) VAS (visual analogue scale)

Overall there is a of lack of validation of patient reported outcome measures in rheumatology and across SpA subtypes.[152] However, the Food and Drug Administration has recommended ≥30% decrease on patient-reported outcomes for abdominal pain be

considered clinically significant in clinical trials for adults, which provides some benchmark for clinical outcomes in this study.

1.7.11 Stiffness

Stiffness is an important indicator of inflammatory musculoskeletal diseases. Morning stiffness was included in the original American College of Rheumatology classification of RA and remains an indicator of inflammatory activity used by rheumatologists for crucial decision-making.[153] Stiffness is thought to be directly related to circadian rhythmic increases over night of pro-inflammatory cytokines such as IL-6 .[154]

Composite scores may contain sub elements relating to stiffness, which will be discussed later, such as the Bath Ankylosing Spondylitis Disease activity Index.[154] When interviewed, patients have outlined the interaction of stiffness with pain, its contextual variability and the impact it has upon function, over and above the quantification of stiffness time in the morning, thus making stiffness a hard aspect to quantify.[155]

The subjectivity and heterogeneity of the concept of stiffness may be why a 'gold standard' assessment that considers life impact of stiffness does not yet exist.[156] A dedicated morning joint stiffness outcome measure has recently been developed and has shown reliability, validity, and responsiveness in the older RA cohort in measuring change in morning duration and severity, suitable for trial outcomes.[157] This is an important area to consider, as out of a survey of 154 patients with RA who had retired, 64% identified RA-related morning stiffness as the key driver for leaving the workforce.[158]

1.7.12 Overall wellbeing

The contribution of disease management to overall wellbeing is assessed through quality of life measures, which are widely recognised as an important component of outcomes in rheumatic diseases.[159] There are a wide variety of measures available, and those commonly used in rheumatology are briefly described below in Table 9.

Table 9 Characteristics of four common patient reported outcomes for quality of life.

OMERACT core area **Life impact**	The Western Ontario and McMaster Universities Arthritis Index (WOMAC)	Patient global assessment (PtGA)	Arthritis Impact Measurement scales (AIMS)	Stanford Health Assessment Questionnaire (HAQ)
Admin	A self-administered questionnaire of 24 items, three subscales: (pain, stiffness and physical function) scored: None (0), Mild (1), Moderate (2), Severe(3) and Extreme(4).[160]	Single question rated by the patient, on a scale of 0 -10cm or 0-100 mm. Higher scores represent a higher level of disease activity [161]	Sections for mobility, physical activity dexterity, household activity social activities, daily living, pain, anxiety and depression.[162]	Self-administered questionnaire covering 5 domains (death, disability, discomfort, iatrogenic and economic aspects)
Scoring	Scores for each subscale are summed to give a total. Higher scores indicate poorer health	VAS format may vary from an unmarked line, divisional ticks or a Likert scale.[161]	Higher scores indicate poorer health.	Higher scores indicate greater disability.[164] A range of scoring methods used .
MCID	MCID ranged from 0.51 to 1.33 points (scale 0 to 10) in post-surgical patients .[163]	Unstated – may be considered similar to other utilities of the VAS scale.	Standardised response means range from 0.36 (small) to 0.8 (high).	clinically important improvement in HAQ (defined as ≥ -0.22).[164]
Reliability Test retest	varies for the different subscales so should stipulate the rating and scoring methods applied.[160]	Acceptable to high [161]	AIMS correlations between 2 administrations over a 2-week period 0.80.	ranged from 0.87 to 0.99 For the Dimension related to physical function.[165]
Internal	high internal consistency rating (> 0.7).[166]		AIMS2. vary over the 9 sections range from 0.72 to 0.91.[167]	internal consistency of 0.78–0.84 in patients with SpA.[165]
Development	Developed for the evaluation of hip and knee osteoarthritis.	Originally designed for the assessment of pain in RA	Developed for Osteoarthritis.[162]	Developed in 1980 for all forms of arthritis.
Pop norms/	Population-based age- and gender-specific normative values are available.[168]	Not stated but ACR/EULAR remission criteria use PtGA <1.[169]	none	32% of an older population report some disability when assessed with HAQ,[170]
Construct validity	validity shown with other outcomes measure for impairment and disability .[166]	research suggests pain, functional incapacity, and fatigue are the strongest factors. [171,172]	AIMS2 has internal consistency and moderate correlation with levels of disease activity, pain scales and ESR.[162]	The HAQ-Disability Index significantly correlated with other measures of self-report.[165,173]
versions	Available in 5-point Likert, 11-point numerical rating, 100-mm visual analogue scale (VAS), digital and LOTE versions.	Heterogeneity of wording creates a variety of informal versions. i.e. PtGA of disease activity.[174]	AIMS2-SF assesses five components of health status AIMS2 also includes arm function.[175]	Different scoring applies to the short.[176] .[165]
Limitations/comments	Scale may be insensitive if the link between pain and function is weak, as may occur in more chronic conditions and confounded by physical disability.	Lacks face validity when used alone. Poor correlation with DAS28, Poor agreement with doctors rating.[177-179]	A broad scope tool, so potential to be impacted by many other elements in longstanding disease.	Clarity of version and scoring is essential for the interpretation of HAQ results

*ACR (American College Rheumatology) AS (Ankylosing spondylitis) IBS(Irritable bowels syndrome)ESR (Erythrocyte sedimentation rate) EULAR(European Union League Against Rheumatism) LOTE (language other than English) MCID(Minimal clinically important difference) QoL(Quality of life) RA (rheumatoid arthritis) SF-26 (Short form 36) SpA (Spondylarthritis) VAS(visual analogue scale)

Many measurement tools exist in varying versions which can impact administration and interpretation of results. There is a viewpoint that given the heterogeneous nature of inflammatory conditions, and unique symptoms affiliated with specific diagnoses, that generic measures are insufficiently sensitive to provide patient centred quality of life outcome data. Some condition specific patient reported outcome measures are briefly discussed below in Table 10.

Table 10 Characteristics of three disease specific patient reported outcomes for quality of life.

OMERACT core area Conditions specific		**Bath AS Functional Index** BASFI	**Bath AS Disease Activity Index** BASDAI	**Ankylosing Spondylitis Quality of Life** AsQol
Admin		Self-administered 10 questions, rated 0-10 VAS or Numerical Rating scale (NRS) that focus upon functional capacity to for everyday tasks.[180]	Six questions rated on a VAS or NRS scale from 0 (non-problem) through to 10 (worst problem).[180]	18 dichotomous items forming a single scale. Includes impact on sleep, mood, motivation, coping, activities of daily living, independence, relationships, and social life. [181]
Scoring		Scores are summed and then divided by ten to give an average. Higher scores indicate greater the functional impairment.	Two questions rate stiffness which are averaged before final score calculation.	No scored zero, YES scored 1 Total score is the sum of the individual scores. Higher scores reflecting greater impairment of health-related quality of life.
MCID		MCII was 0.6 for the BASFI.[182]	MCII was 1.1 for the BASDAI.[182]	Information not available
reliability	Test retest	intraclass correlation coefficient reported a 0.89 to 0.92 [181]	intraclass correlation coefficient of 0.87 [183]	retest reliability (rs=0.92 and rs=0.91[181]
	Internal	0.936 [183]	of 0.84–0.87 [183]	between 0.89–0.92 [181,183]
Development		Designed specifically for patients with AS. They include the Bath AS Metrology Index (BASMI), the Bath AS Functional Index (BASFI), the Bath AS Disease Activity Index (BASDAI) and the Bath AS Patient Global Score (BAS-G).[184]		Developed to look at QoL from the AS patient's perspective.[181,185]
Criteria validity		Been investigated for use in other spondyloarthritic conditions, with preservation of content validity and measurement properties.[184,186,187] Bath indices correlates well with other AS-specific health outcome measures and sensitive to change at all disease levels.[188]		Correlates moderately well with other AS-specific health outcome measures .[183] Applicable AS and Nr.Ax. SpA .[181] [185,189]
Limitations/comments		Validation studies positive but conducted some years ago, for patients in a steady state or remission.[185] Bath Indices do not include any objective measures or clinician's perspective.		Most frequently used disease-specific measure of health-related quality of life in AS studies

* AS (Ankylosing spondylitis) ESR (Erythrocyte sedimentation rate) MCII (Minimal clinically important improvement) QoL (Quality of life) NRS (numerical rating scale) Nr.Ax. SpA (Non radiographic Spondyloarthritis) RA (rheumatoid arthritis) SF-26 (Short form 36) SpA (Spondyloarthritis) VAS (visual analogue scale).

1.7.13 Resource use/economic impact

All the conditions included in this review are chronic conditions and are associated with the likelihood of progressive disability. Increasing disability may create a declining quality of life and often incurs significant costs to both the individual and society. The costs of inflammatory forms of arthritis are significant. For example the estimated costs of biologics for RA, as a single disease entity, in the 2014-2015 financial year were approximately AUS $273 million in Australia according to the Counting the Costs report by Arthritis Australia, which represents a 104% increase from the costs reported in 2007.[190] Taking into account the costs for biological disease modifying anti- rheumatic medications, healthcare costs for RA were estimated to be over AUS $550 million by 2030, representing a AUS $102 million increase from 2015 costs".[190 (p.24)] Whilst the development of 'biosimilar' medications may reduce these estimates, the costs remain significant and do not include the wider costs, for example loss of earnings from reduction in work hours or early retirement due to the disease, disability payment costs, and other non-pharmaceutical interventions such as surgery, to name but a few. This may seem to make a clear case for the economic evaluation of alternative interventions.

Economic evaluation often employs one of four methods: cost minimisation analysis, cost effectiveness analysis, cost utility analysis, and cost benefit analysis. These are scientifically based ways of allocating resources for health interventions and can only be employed when the benefits and efficacy of an intervention from a clinical perspective are well established. Therefore, it is required that the efficacy of specific probiotic strains and formulations is first established, prior to embarking on economic outcomes that may guide real world decision making and accessibility for patients.

1.8 Current state of evidence, and justification for review

1.8.1 Justification of approach

This Master's thesis has employed the application of a systematic review in order to provide guidance to clinicians and consumers regarding the efficacy of a specific health intervention to a specific population, that of probiotics for individuals living with an inflammatory arthritis. Evidence-based medicine involves the "explicit, conscious and judicious attempt to find the best available evidence to assist health professionals".[191(p.71)] Whilst there is consensus that modern healthcare should be informed by the best available evidence in

order to maximise safety, quality and health outcomes for patients, keeping up to date with the rapid evolution of medical knowledge and overwhelming volume of published articles is problematic. It has been estimated that more than 2 million articles per year, are published in over 20,000 journals.[192] Therefore in reality, individuals may form opinions regarding the efficacy of interventions that are based on limited or biased sources of information, and therefore deliver misrepresentations of estimates of benefits and harms.[193]

Understanding what has already been studied in any given health field by undertaking a literature review has been an established manner of sourcing information. A literature review collates a range of papers in a current field, with discussion and judgement delivered in a narrative form by the authors. Whilst this helps readers to make decisions by drawing together relevant results and data in a single publication, literature reviews lack a systematic approach to the key processes of scoping, analysis, data synthesis and appraisal of bias. This deficit can create reviews with heterogenous studies, at risk of type I and type II errors as well as hidden bias. This Masters utilises a SR approach, conducted with the rigor expected of the studies contained within it.[194] SR aim to address a specific question, using systematic and explicit methods to identify, select, and critically appraise relevant studies. Whilst an SR does collect and analyse data from included studies, it does not seek to create new knowledge but rather to synthesise and summarise existing knowledge.[194(p55)] The value of SRs is demonstrated by their place at the top of the hierarchical pyramid of evidence. SR are conducted world-wide by a number of organisations for example the Cochrane Collaboration - an organisation mainly focused on the effectiveness of health interventions from randomised controlled trials and other groups such as the Joanna Briggs Institute (JBI) (http://joannabriggs.org) which include other study designs and types of evidence in their systematic reviews.[195]

The key potential weakness of a systematic review process are outlined and include " a lack of systematic and transparent conduct and reporting, …unrecognised and unaccounted statistical and clinical heterogeneity, data dredging in non-predefined statistical analyses, and a lack of assessment of the overall quality of evidence."[196 (p.518)] To address such concerns this thesis applied JBI methodology.[197] The JBI is part of the Faculty of Health and Medical Sciences, University of Adelaide and has been working to develop and promote

evidence-based practice since 1996. They support the employment of rigorous review methodology including the utilisation of preferred reporting items for systematic reviews, known as PRISMA and the GRADE methodological approach for rating the quality of evidence within a review.[198-200] This ensures a further rigor to the SR process by considering study design, risk of bias, precision, consistency, directness, publication bias and magnitude of effect in a transparent and standardised fashion.[199,200]The JBI has recently updated their evidence based model of healthcare, to recognise the role of evidence informing a more complex shared decision process between patients and clinical experts. This update of the JBI model recognises the contribution of systematic reviews and statistical facts but also the judicious use of expert knowledge, patient real-life priorities and the need for simple tools that outline risk and benefit to enable open dialogue between health professionals and patients. This thesis aims to deliver a SR that can be used within the wider paradigm of the Evidence Informing Healthcare Model.[201]

1.8.2 Current research in the field

A preliminary search of the Cochrane Library, JBI Database of Systematic Reviews and Implementation reports (JBISRIR), PubMed, CINAHL, and PROSPERO was conducted for existing systematic reviews related to this topic from Feb to April 2017. Four relevant systematic reviews were identified, which explored the benefits of probiotics for RA alone, in adults, with a range of outcomes examined.[202-205]

Pan et al[202] and Rudbane et al[203] both focused on changes in systemic inflammation outcomes (Disease Activity score S28, C-Reactive protein and cytokine expression) including five and six randomised controlled trials, respectively, with full overlap between reviews. All patients had a classification of RA. Pan et al[202] concluded that whilst changes in systemic inflammatory outcomes were observed they did not meet the American College of Rheumatology criteria for 20% improvement and Rudbane et al[203] concluded the statistically significant improvements seen may not reach clinical significance. Mohammed et al 2017[204] revised the inclusion criteria and found nine studies (randomised controlled trials and quasi-experimental trials) for patients with RA, analysed similar systemic inflammation outcomes and came to similar conclusions. Whilst changes in cytokine biomarkers were identified their significance was undecided.[204] The review by Dejoras et al [205] was lacking detail as published

in an abstract form, but used similar databases, population group and study inclusion criteria to the other reviews.[205] Dejoras et al[205] concluded again that potentially beneficial changes in systemic inflammation markers were seen in trials but that larger study sizes were required.

A review by Mazidi et al[206] concentrated on the changes in C-reactive protein alone, and included three studies of rheumatology patients amongst a total of 20 included studies. With a greater number of trials to analyse they concluded that probiotics may significantly reduce C-reactive protein, but not other biomarkers.[206]

Whilst Didari et al[44] have undertook a review on safety of probiotics, there were no studies on patients with rheumatic conditions were included. This systematic review aims to contribute to the growing literature of the potential effectiveness and safety of probiotics as a sole or adjuvant therapeutic intervention for individuals with inflammatory arthritis. Systematic reviews were not found which were solely focused on the Spondyloarthopathies, despite their arguably closer links with gut dysbiosis and pathology. This systematic review aims to contribute to the growing literature of the potential effectiveness and safety of probiotics as a sole or adjuvant therapeutic intervention for individuals with inflammatory arthritic disease by including a wider range of conditions. This will allow for the heterogeneous overlapping nature of inflammatory disease and enable individuals with earlier disease states to be included, providing clearer guidance to clinicians in daily practice by increasing the transferability of findings.

The outcomes of the systematic reviews discussed above, were divided across a range of outcome markers but primarily clinician reported outcomes and surrogate markers. None of the systematic reviews noted investigated patient reported outcomes, fatigue, bowel symptoms or other outcomes that impact quality of life. Most of the reviews restricted inclusion to randomised controlled trails, thereby limiting evidence to studies with established disease states able to meet more stringent disease classification criteria. This potentially excludes the patient population most likely to engage with probiotics use and missing the early window of opportunity in which intervention may be most effective. It must be recognised that to direct clinical intervention and patient counselling regarding the use of probiotics, more data on risks and harms specific to rheumatology is required. This

review aims to add to the knowledge base by including adverse effects of probiotics as a primary outcome.

Studies into clinician's views on their patients using probiotics in rheumatology in Australia have not been formally undertaken. However, it is known from studies on other complementary medicines that knowledge of the therapy and level of scientific rigour applied to assessing it, are important factors that affect clinician's willingness to consider alternative interventions.[207,208] Therefore this review aims to inform clinicians and contribute to the rigorous evidence base around probiotics.

Most importantly, none of the systematic reviews that include patients with rheumatic diseases provided subgroup analysis or discursive analysis on the preparations and compositions of probiotic supplied. Therefore, this review aims to match emerging science on strain-specific benefits to the specific formulations in each study along. The review will also apply strict definitions of probiotics and undertake greater consideration of important factors such as concentration, inclusion of prebiotics, and encapsulation methods that have been used within a wider variety of quantitative study designs. Whilst it is recognised that there are many challenges in undertaking a review with strain-specific analysis, it is increasingly recognised that this is paramount if clinicians are to prescribe probiotics appropriately.[209]

Whilst research on probiotics for gastroenterology is more established than for rheumatology, it is only in 2018 that a systematic review by McFarland et al.[209] has used a strain-specific approach to make recommendations on efficacy of probiotics for adult antibiotic associated diarrhoea. This review discovered that certain strains of Lactobacillus were more effective than others in managing adult antibiotic associated diarrhoea and that past systematic reviews and meta-analyses were poorly placed to identify this.[209,210] Applying this strain-specific approach to the probiotic interventions used for rheumatology may provide the clarity required to identify probiotics with appropriate therapeutic potential in this area.

CHAPTER TWO: Systematic review methodology

2.1 Review objective

The objective of this review was to identify the reported strain specific effectiveness and the strain specific adverse effects of probiotics when used as a therapeutic intervention for individuals living with inflammatory arthritis.

2.2 Criteria for studies to be included

2.2.1 Inclusion criteria: Types of participants/population of interest

This review considered studies that include individuals (adult or child) of any gender living with the following specific forms of diagnosed inflammatory arthritis:

- Rheumatoid arthritis
- Adult spondyloarthritis (of any form)
- Juvenile arthritis (of any form)

Individuals with early, established or severe forms were included. Study participants should have been diagnosed for a minimum timeframe of three months. Diagnosis was used rather than clinical classification as whist this may lower specificity it increases generalizability of study finding for real life clinical practice.

2.2.2 Inclusion criteria: Types of intervention

This review *included* studies that:

- Supply probiotics that meet the definition of probiotics as defined in 2001 by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organizations (WHO) "live microorganisms which, when administered in an adequate amount, confers a health benefit to the host".[33]
- Evaluate probiotics administered to the gut (either orally or via enteral feed).
- Utilise any concentration of probiotics, measured in colony forming units.
- Employ any strain or species, or combination of species. Probiotics may be administered with prebiotics (thereby termed a synbiotic). Products that do not require refrigeration utilise spore-forming bacteria are included as whilst the spores are dormant, there is evidence to suggest that these spores can germinate within the gut and exhibit benefits.[211]

This review *excluded* studies that:

- Utilise prebiotics alone as these microbial cultures may deliver useful enzymes but have not been shown to proliferate and alter the microbial composition of the gut.[33]
- Utilise probiotics within functional foods or fecal matter transplants as they include many microbial species in unknown and unstandardised quantities.[37]

2.2.3 Inclusion criteria: Types of comparator(s)

Studies that utilised probiotics as a sole or adjuvant therapeutic intervention to standard care, compared to standard care alone, are considered.

2.2.4 Inclusion criteria: Types of outcome

This review considered studies that include any outcomes specified in the core set of appropriate measures by the international consortium known as OMERACT (Outcome Measures in Rheumatoid Arthritis Clinical Trials).[90,213] Primary outcomes included:

A. Single measure patient reported outcomes, including but not limited to, Patient Global Assessment, Pain, Fatigue and Health Assessment Questionnaire.
B. Composite measures of patient reported outcomes, for example;
   the short Health Assessment Questionnaire which is based on three patient centered dimensions: pain, patient global assessment and disability.
C. Composite indices that include outcomes from patient, provider and a laboratory-based score of inflammatory markers, for example, the Disease Activity Score.

Composite indices that include surrogate lab markers for inflammation were included as a core part of all major guidelines and as accepted quality indicators for effective management. It is recognised that they may be affected by the weighting of specific elements that and this is considered in the results analysis.

D. Safety and patient reported adverse effects were considered including
   minor reported outcomes, such as gastrointestinal upset or flatulence, and major reported outcomes, such as sepsis, interaction with concurrent standard care/medication or mortality.

2.2.5 Inclusion criteria: Types of study

A range of experimental and epidemiological study designs including randomised controlled

trials, non-randomised controlled trials, quasi-experimental studies, prospective and retrospective cohort studies, case control studies and analytical cross-sectional studies were eligible for inclusion in this review. Also, descriptive epidemiological study designs including case series, individual case reports and descriptive cross-sectional studies were eligible for inclusion. Studies that were published in English on humans were eligible for inclusion.

Head to head studies, which compared interventions rather than use one intervention against control were included. The findings of head to head studies were treated with caution as extrapolating the comparison of two interventions whereby there is a current absence of firm data on the reliability and safety of either is not recommended.

2.3 Method of the review

This systematic review was carried out in accordance with the published protocol in the JBI Database of Systematic Reviews and Implementation reports and Prospero centre for Reviews and dissemination (CRD42019122116).[212]

2.3.1 Search Strategy

The search strategy aimed to find both published and unpublished studies. Studies published from 2000 were considered for inclusion in this review as probiotics were clearly defined at this time. A three-step search strategy was utilised in this review. Firstly, an initial limited search of MEDLINE and CINAHL was undertaken followed by an analysis of the text words contained in the title and abstract, and of the index terms used to describe the article. For each database specified, the search terms and their related terms were expanded to cover relevant variations and database specific terminology or abbreviations in order to identify relevant search terms that reflected the studies inclusion criteria. The key word employed in this preliminary search therefore, identified the population with inflammatory arthritis, (rheumatoid arthritis, psoriatic arthritis, auto immune arthritis and juvenile arthritis), of any age, taking the intervention (probiotics, synbiotics) in clinical trials with any outcome.

A second search using all identified keywords and index terms was then undertaken across all included databases. It included search terms specific and related to probiotics and the designated inflammatory arthritis conditions. Thirdly, the reference list of all identified reports and articles was searched for additional studies. Only studies published in English

were considered for inclusion in this review as this was the first language of the reviewers, due to resource constraints as relates to the provision of translation services and research indicates no evidence of a systematic bias from the use of language restrictions in systematic review-based meta-analyses in conventional medicine.[214]

Initial keywords

Probiotics, Synbiotics, Rheumatology, Arthritis

The databases searched included: *Pubmed, CINAHL, EMBASE, SCOPUS*
The trial registers searched:

- The Cochrane central register of controlled trials (CENTRAL)

  The World Health Organization clinical trials portal (ICTRP)
- NIH clinical trials register Clinicaltrials.gov
- Australian New Zealand Clinical trials register (ANZCTR).

The search for unpublished studies included:

- Pro dissertations and theses (PQDT)
- Bielefeld Academic search engine (BASE)
- System for Information on Grey Literature in Europe (OpenGrey)
- Health Services Research Projects in Progress (HSRProj)
- Mednar Deep web search technology database

Due to the large volume of published literature in this field the retrieved articles were exported to a reference management system (Endnote) and screened in a three-step process. First by title, then by abstract and finally full text screening to ensure all articles met the inclusion criteria. For example, full text screening enabled the identification of studies that employed the application of bacterial cell wall components as opposed to live probiotics or utilised probiotics contained within functional foods in unspecified quantities.

Pub Med Search terminology

Condition Descriptors relevant to Inflammatory arthritis

(Arthritis[mh] OR Arthritis[tw] OR JIA[tw] OR Enthesitis[tw] OR Polyarthritis[tw] OR Rheumatoid[tw] OR Psoriatic[tw] OR Rheumatoid Arthritis[mh] OR Rheumatoid arthritis[tw] OR Rheumatic disease[tw] OR Rheumatism[tw] OR Spondyloarthritis[mh] OR Spondyloarthropathies[mh] OR Spondyloarthr*[tw] OR Ankylosing Spondylitis[mh] OR

Ankylosing Spondylitis[tw]) OR enteropathic arthritis[tw] OR Arthralgia[mh] OR Arthralgia[tw])

Intervention descriptors relevant to probiotics

(Probiotics[mh] OR Probiotic*[tw] OR Synbiotics[mh] OR Synbiotic*[tw] OR Microbiota[mh:noexp] OR Microbiota[tw] OR Gastrointestinal microbiome[mh] OR Gastrointestinal microbiome[tw] OR Microbiome[tw] OR Gut microbiome[tw] OR Dysbiosis[mh] OR Dysbiosis[tw] OR Gut Flora[tw] OR Gut microflora[tw] OR Gastrointestinal flora[tw] OR Gastrointestinal microflora[tw] OR Lactobacillus[mh] OR Lactobacill*[tw] OR Bifidobacterium[mh] OR Bifidobacter*[tw] OR Saccharomyces[mh] OR Saccharomyces[tw] OR Escherichia[mh] OR Escherichia[tw] OR Bacillus[tw] OR Bacillus[mh] OR Dietary supplement[mh] OR Dietary supplement*[tw] OR Food supplement*[tw] OR Diet therap*[tw] OR Nutrition therapy[mh] OR Nutrition therap*[tw] OR Nutritional therap* OR Nutraceutical*[tw] OR Nutriceutical*[tw] OR Neutraceutical*[tw])

Table 11 Core search terminology applied regarding Population and Intervention

Population Condition	**AND**	**Intervention-**
(Arthritis[mh] OR Arthritis [tw] OR JIA[tw] OR Enthesitis [tw] OR Polyarthritis [tw] OR Rheumatoid [tw] OR Psoriatic [tw] OR Rheumatoid Arthritis[mh] OR Rheumatoid arthritis [tw]OR Rheumatic disease [tw] OR Rheumatism [tw]OR Spondyloarthritis [mh] OR Spondyloarthropathies [mh] OR Spondyloarthr*[tw] OR Reactive arthritis [tw] OR Enteropathic arthritis [tw] OR Inflammatory Bowel disease [mh] OR Inflammatory Bowel disease [tw] Arthralgia [tw] OR Ankylosing Spondylitis[mh] OR Ankylosing Spondylitis [tw])		(Probiotics [mh] OR Probiotic*[tw] OR Synbiotics [mh] OR Synbiotic* [tw] OR Microbiota [mh:noexp] OR Microbiota [tw] OR Gastrointestinal microbiome [mh] OR Gastrointestinal microbiome [tw] OR Microbiome[tw]OR Gut microbiome[tw]OR Dysbiosis[mh] OR Dysbiosis[tw] OR Gut Flora [tw] OR Gut microflora [tw]OR Gastrointestinal flora[tw]OR Gastrointestinal microflora[tw] OR Lactobacillus [mh] OR Lactobacill*[tw] OR Bifidobacterium[mh] OR Bifidobacter*[tw] OR Saccharomyces [mh] OR Saccharomyces [tw] OR Escherichia [mh] OR

		Escherichia [tw] OR Bacillus [tw] OR Bacillus[mh] OR Dietary supplement [mh] OR Dietary supplement* [tw] OR Food supplement* [tw] OR Diet therap* [tw] OR Nutrition therapy[mh] OR Nutrition therap*[tw] OR Nutritional therap* OR Nutraceutical* [tw] OR Nutriceutical*[tw] OR Neutraceutical*[tw])

CINAHL Search Terminology

((MH “Arthritis”) OR “Arthritis” OR “JIA” OR “Enthesitis” OR “Polyarthritis” OR “Rheumatoid” OR “Psoriatic” OR MH “Rheumatoid Arthritis” OR “Rheumatoid arthritis” OR “Rheumatic disease” OR “Rheumatism” OR MH”Spondyloarthritis” OR MH”Spondyloarthropathies” OR “Spondyloarthr*” OR MH”Ankylosing Spondylitis” OR “Ankylosing Spondylitis”) AND (MH "Probiotics") OR “probiotic” OR “ Synbiotic” OR (MH "Prebiotics") OR (MH "Lactobacillus") OR (MH "Lactobacillus Acidophilus") OR Saccharomyces[tw] OR (MH "Bacillus") OR (MH "Escherichia") OR (MH "Gram-Negative Bacteria") OR (MH "Bifidobacterium") OR "synbiotics" OR (MH "Microbiota") OR (MH "Gut Microbiota") OR "microbiome" OR (MH "Dietary Supplements") OR (MH "Dietary Supplementation") OR "nutraceutical" OR (MH "Enteral Nutrition")

Embase Search terminology

('arthritis':de,ti,ab OR 'jia':ti,ab OR 'juvenile idiopathic arthritis':ti,ab OR 'juvenile chronic arthritis':ti,ab OR 'reiter syndrome':ti,ab OR 'enthesitis':ti,ab OR 'polyarthritis':ti,ab OR 'arthritis, rheumatoid':de,ti,ab OR 'rheumatic disease':ti,ab OR 'rheumatism':ti,ab OR 'psoriatic arthritis':de,ti,ab OR 'reactive arthritis':ti,ab OR 'post-infectious arthritis':ti,ab OR 'spondylarthropath*':de,ti,ab OR 'autoimmune arthritis':ti,ab) AND ('probiotic':de,ti,ab OR 'synbiotic':ti,ab OR 'microbiota':de,ti,ab OR 'gastrointestinal microbiome':de,ti,ab OR 'microbiome':ti,ab OR 'gut flora':ti,ab OR 'gastrointestinal flora':ti,ab OR 'gastrointestinal microflora':ti,ab OR 'lactobacillus':de,ti,ab OR 'bifidobacterium':de,ti,ab OR

'saccharomyces':de,ti,ab OR 'escherichia':de,ti,ab OR 'bacillus':de,ti,ab OR 'dietary supplement':de,ti,ab OR 'food supplement':de,ti,ab OR 'diet therapy':ti,ab OR 'nutrition therapy':de,ti,ab OR 'nutritional therapy':de,ti,ab OR 'nutraceutical':de,ti,ab OR 'nutriceutical':ti,ab OR 'neutraceutical':ti,ab) AND [english]/lim AND [2000-2018]/py AND [embase]/lim

Scopus

(arthritis OR (“juvenile arthritis") OR (" reiter AND syndrome") OR enthesitis OR rheumatoid OR ("rheumatic disease") OR rheumatism OR ("psoriatic arthritis") OR ("reactive arthritis") OR spondyloarth* OR ankylosing OR arthralgia) AND (probiotic OR synbiotic OR microbiota OR ("gut flora") OR ("Gut microflora") OR lactobacill* OR bifidobacteri* OR saccharomyces OR escherichia OR bacill* OR ("gastrointestinal microbiome") OR ("gastrointestinal flora") OR ("Dietary supplement*") ("food supplement*") OR ("nutrition therapy") OR nutr?ceutical* OR ("diet therap*") OR "nutrition* support")

2.3.2 Assessment of methodological quality

Papers selected for retrieval were assessed by two independent reviewers for methodological quality prior to inclusion in the review using standardised critical appraisal instruments from the JBI.[207] Any disagreements that arise between the reviewers was resolved through discussion, or with a third reviewer.

All articles were included within the review, and quality appraisal was included within the narrative discussion of results. No specific threshold for inclusion of studies was applied as risk of bias assessments are undertaken within the GRADE approach and accounted for before any clinical recommendation or systematic review conclusions are made.

2.3.3 Data extraction

Quantitative data was extracted from papers included in the review using the a tailored excel spread sheet that accommodated specific information relevant to the study of probiotic interventions. The extracted data included specific details about the interventions, populations, study methods and outcomes of significance to the review question and specific objectives. Detailed demographic data was mined from the studies as these factors

impact upon the probiotics mechanism and have been poorly considered previously when interpreting data and ensuring clinical transferability of study outcomes. Attention was specifically given to the nature of the probiotic formulation with respect to included bacteria, their taxonomic classification and dosage in colony forming units. Data was extracted wherever numerical results were reported. The authors of included studies were contacted where necessary to request any relevant data that was not available in the published articles. Due to the time constraints of this thesis 6 months response time was allowed.

2.3.4 Data synthesis

Data were grouped by outcome that matches the key OMERACT outcome domains, regarding adverse events (from minor to major), quality of life (patient reported outcomes with respect to pain, fatigue, general wellbeing and bowel symptoms) and markers of systemic inflammation (C-reactive protein and disease activity score). These outcomes were cross referenced to the demographic and probiotic formulation data extracted from the studies. A table was presented to summarise the study characteristics of included trials and is provided in chapter 4. Results of all included studies across all included outcomes were synthesised in narrative form with the inclusion of tables and graphics to aid in data presentation. Meta-analysis was considered where enough studies employed similar outcomes such that a weighted mean difference or standardised mean difference would be appropriate. Where meta-analysis was inappropriate due to heterogeneity or incomparable data, a narrative review was undertaken. A standardised robust approach was taken to perform meta-analysis on the difference between post test scores in the experimental studies (as opposed to mean change) for most outcomes. This necessitated the calculation of post test data in studies where only baseline and change from baseline were reported.

2.4 Statistical analysis

2.4.1 Data conversions

Where studies presented data as median and interquartile ranges, they were converted to mean using the formulas supplied in Weir et al.[215] Where data was presented on the same outcome measure but on different scales (for example pain measured un VAS, or CRP measure in mg or g per L) the data was converted to the most common utilised scale.

2.4.2 Missing data

Where missing or incomplete data was presented in studies, this was first resolved by communicating with the authors to request full raw data. Where this was not achieved and missing data values remained the approach of Weir et al[215] was employed, as their Illustrative meta-analyses showed that "replacing a missing SD by approximation using the range minimised loss of precision and generally performed better than omitting trials."[215(p.1)] Using the baseline values and mean change scores, estimates for post-test scores were achieved, however if the p value for post-test scores was not known the Cochrane handbook methodology was applied, whereby an average of the baseline standard deviation were taken as an approximate of post-test standard deviation.[216]

2.4.3 Effect size calculations

Effect sizes were expressed as relative risk (for categorical data) and weighted mean differences (for continuous data) and their 95% confidence intervals have been calculated for analysis in case of identical scales across studies; otherwise, standardised mean difference (SMD) was used. Effect size was calculated using the difference between post-test scores, where no statistical difference in baseline values exist.

2.4.4 Secondary outcome and incidental findings reporting

Data concerning the outcomes of interest including adverse effects was mined from all studies irrespective of its status as a primary outcome measure. Whilst this may limit the rigor of the reporting, evidence of harm is a major consideration when communicating the risk/benefits of probiotics with patients and therefore this scrutiny is valid.

2.5 Meta-analysis

Any relevant quantitative data was, where possible, pooled in statistical meta-analysis. Meta-analysis performed using System for the Unified Management, Assessment and Review of Information (SUMARI) was be considered for all outcomes. Where more than five studies were available for meta-analysis a random effects approach was chosen as the variation in formulation of probiotics applied was hypothesised to be a key factor influencing outcomes above and beyond sampling error or chance. As random models of calculation provide a more conservative model of effect size this was deemed more appropriate for the heterogeneity of studies included. Where limited studies were included

a fixed approach was taken and, in all cases, the statistical heterogeneity was assessed using X^2 and I^2 test and statistics. Significant heterogeneity was considered when X^2 test has a P value < 0.1 or an I2 test value > 50%.

2.5.1 Subgroup analysis

Results explored using subgroup analyses where appropriate. Where possible subgroup analysis based on condition, and on data for specific strains and combinations of probiotics was be employed. Where statistical pooling is not possible the findings were presented in narrative form including tables and figures to aid in data presentation where appropriate.

2.5.2 Confidence in effect size

A summary of findings table was generated in order to provide a concise outline of key findings for each of the outcomes and to provide an accessible format to outline the certainty of confidence in the effect estimates from the review. The review effect estimates were graded from very low (very little confidence in the effect estimate) up to high (very confident that the true effect lies close to that of the estimate of the effect) after considering the factors that may increase or decrease confidence for each included study. As per the grade methodology factors that may increase confidence included magnitude of effect, dose response gradients and plausible confounders. Factors that may reduce confidence for an individual study included Limitations in study design or execution (risk of bias), inconsistency of results, indirectness of evidence, imprecision and publication bias.[217]

CHAPTER THREE Results

3.1 Study Selection

A total of 6,316 articles were identified from searching the specified databases, and a further 1,842 were identified from grey literature searching including clinical trial databases, dissertation databases and online deep web searches. These article citations were exported into the reference manager software Endnote (Clarivate Analytics), and after removing duplicates, 5,876 records remained. These were screened for inclusion by title, and after removing 5,278 records deemed not to meet inclusion criteria, 598 records remained. These records were further screened by abstract providing 152 articles which were obtained in full form. Full texts of these 152 articles were retrieved and assessed against the exclusion noted as per appendix IV. Following screening of the reference lists, two further articles meeting inclusion criteria for the review were identified. Of the 154 articles that were reviewed, a final 12 studies met inclusion criteria and were obtained in full form.[218-229] Of these, ten were randomised controlled trials of which nine employed a placebo control methodology and one compared to standard treatment. Two articles were quasi-experimental and only one of these employed a placebo. There were no studies included which provided an active control of an alternative probiotic formulation. Two of the included 12 studies, Alipour et al[218] and Vaghref -Mehrabany et al.[227] were reporting different outcomes for the same trial, and therefore this was accounted for when analysing outcomes to prevent duplication of data. Four studies included a variety of Spondyloarthopathies .[219,221,225,226] Eight studies focused on RA.[218,220,222-224,227-229]OF these the majority employed American College of Rheumatology criteria for RA. Probiotics were supplied for 56 to 84 days in 77% of studies. Overall, 17 different probiotics were supplied in colony forming units ranging from 1x 10^8 to 2.25 x 10^{11} per 24hrs . Lactobacillus was most commonly supplied (59%) then Bifidobacterium (20.8%).

Search and study selection results were presented according to the PRISMA guidelines as shown in Figure 7.[239]

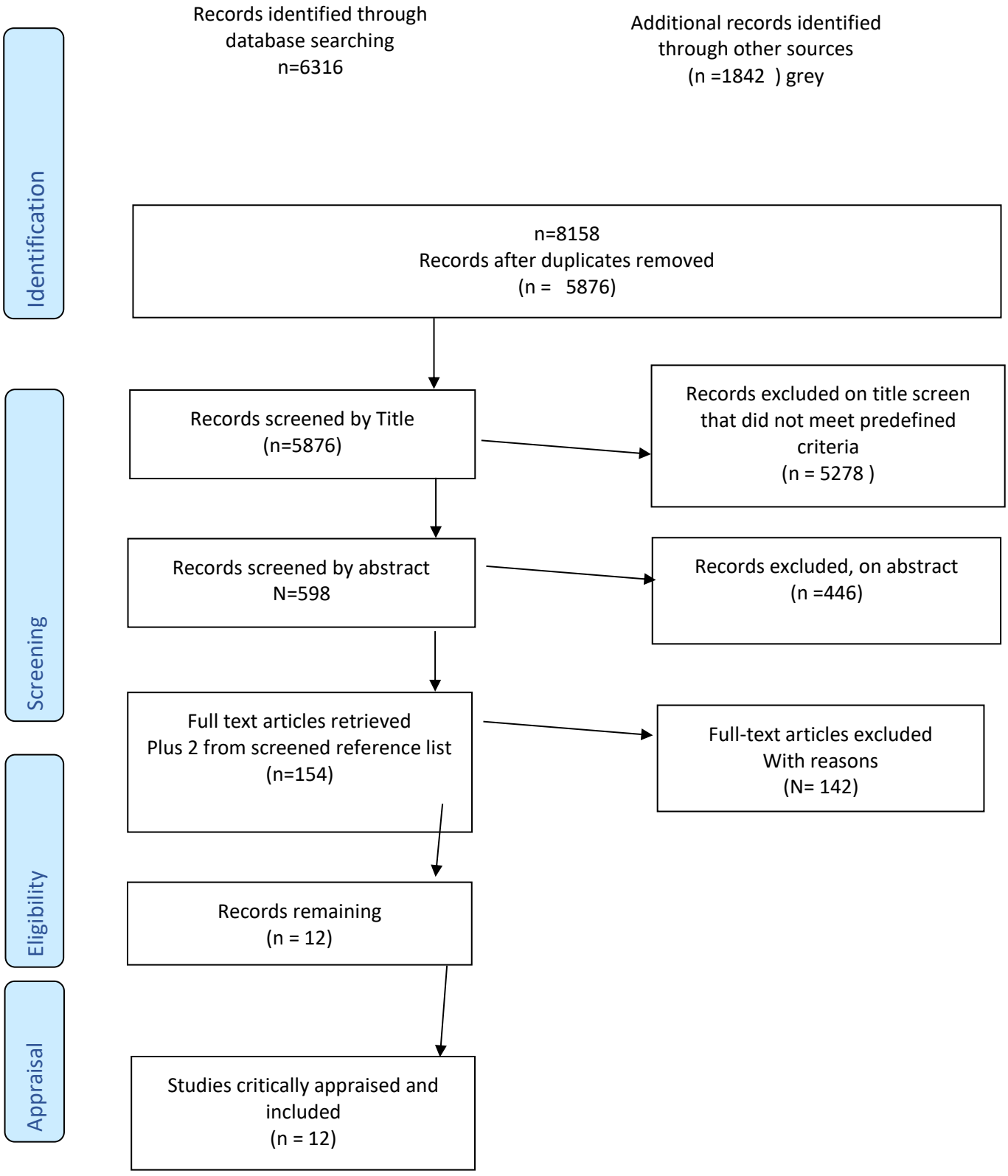


Figure 7 Prisma flow diagram[230]

3.2 Methodological quality

Of the included articles, ten studies were blinded randomised control trials and their quality was appraised using the JBI critical appraisal tool for randomised controlled trials (appendix Ia & Ib). Two reviewers appraised the articles and any disagreements were resolved through discussion and the input of a third reviewer where necessary. The results are shown below in Table 12. Two studies were considered quasi-experimental and their appraisal was completed using the JBI appraisal checklist for quasi-experimental studies (appendix IIa and IIb) and results are shown in Table 13. From the quasi-experimental articles, Tomasello et al.[226] compared probiotics to standard therapy, and Lee et al.[222] provided the probiotic intervention without a control group.

Table 12. Assessment of methodological quality of included randomised control trials.

Study	Question number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Alipour et al 2014[218]	Y	U	Y	Y	Y	Y	Y	U	U	Y	Y	Y	Y
Brophy et al 2008[219]	Y	Y	Y	U	Y	Y	Y	Y	Y	U	N	Y	Y
Hattaka et al 2003[220]	Y	U	Y	Y	U	Y	Y	N	N	Y	Y	U	Y
Jenks et al 2010[221]	Y	Y	Y	Y	U	Y	Y	Y	Y	Y	Y	Y	Y
Mandel et al 2010[223]	Y	Y	U	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
Pineda et al 2011[224]	Y	U	Y	Y	Y	U	Y	Y	Y	Y	Y	Y	Y
Shukla et al 2016[225]	Y	Y	Y	Y	Y	Y	Y	Y	U	Y	Y	Y	Y
Vaghref - Mehrabany et al 2014[227]	U	U	Y	Y	Y	Y	Y	Y	N	Y	Y	U	Y
Zamani et al 2016[228]	Y	U	Y	Y	Y	Y	Y	U	Y	Y	Y	Y	Y
Zamani et al 2017[229]	Y	U	Y	Y	Y	Y	Y	U	Y	Y	Y	Y	Y

Table 13 Assessment of methodological quality of included quasi-experimental studies

Study	Question number								
	1	2	3	4	5	6	7	8	9
Lee et al 2010[222]	Y	NA	NA	N	Y	Y	NA	Y	Y
Tomasello et al 2015[226]	Y	U	Y	Y	Y	U	Y	Y	U

True randomisation was clearly described and employed in nine out of ten studies. Only one study was unclear as to the exact methodology.[227] As this study used alternative outcome data from the trial by Alipour et al.[218] the lack of clarity may be a change in write-up style. Therefore, all included the randomised controlled trials had a secure method to equally distribute potentially confounding variables between study groups.

Allocation of concealment to groups was poorly detailed in two thirds of randomised controlled trials potentially allowing studies to be affected by allocation bias.[231] Whilst true randomisation should ensure baseline population comparability, the studies included in this review involved small populations and inconsistent levels of allocation concealment. Therefore, it was important that populations were consistently similar at baseline in all included studies as this enabled the use of post-test mean differences to estimate the effects size of the intervention.

Unlike some complementary therapies the provision of probiotics can be easily concealed by providing sham capsules providing that all participants, providers of care and assessors are suitably blinded. Five of the nine studies scored the maximum for blinding across all areas, the other four studies did not clearly specify the blinding employed for one out of the three groups in question. All studies ensured participants within the intervention and control/placebo groups were treated similarly. Where participants required any concurrent intervention, this was clearly stated, as the requirement for additional medications (whether steroids, anti-inflammatories or DMARDS) would clearly affect outcomes.

Only half the included randomised controlled trials clearly described complete follow up and the application of intention to treat analysis. Outcome measures were applied consistently and reliably in most studies. Only the study by Brophy et al[219] relied purely upon self-report as it was an online format. This provides a significant flaw in the study with the largest sample size. Whilst it was deemed that appropriate statistical tests were employed, there were a great variety of different statistical methods applied which further validates the choice of a consistent post-test analysis of effects size across all possible studies. All included randomised controlled trials were appraised to have followed appropriate trial design. Failure to analyse participants in the treatment group to which they were allocated

was the major weakness identified across the assessment of methodological quality for the included randomised controlled trials.

Appraisal of methodological quality of the quasi-experimental studies revealed that all clearly identified cause and effect, and all employed multiple post intervention outcome assessments. Two studies provided a suitable control and ensured similar care between groups. Comparison is hard to achieve in quasi-experimental studies, due to heterogeneity of design. Lee et al.[222] did not employ a control group, Shukla et al.[225] compared to placebo and Tomasello et al.[226] compared probiotic intervention to standard care.

3.3 Study characteristics of included trials

A full table outlining the study characteristics of included trials is found in the appendix III The studies were published from 2003 to 2017. A total number of 603 participants were enrolled to treatment and control groups in the eligible 12 studies. As the same population was utilised in two publications (Alipour et al.[218] and Vaghef-Mehrabany et al.[227]), but different outcome measures were provided in each publication, this population of participants was only counted once when analysing participant numbers.

3.4 Description of included studies

3.4.1 Study demographics

Robust clinical studies ensure a study population that is sufficiently homogenous to control known confounding variables but through this process may introduce limitations on the generalizability of results to the broader clinical population. By examining the study demographics carefully, confidence on the transferability of results can be assessed. There are many known variabilities that can affect the microbiome (such as gender, age, diet and concurrent medications) which have been poorly considered in past reviews. This may underly the different conclusions drawn regarding probiotic treatments efficacy in trials and potential effectiveness in clinical practice. Baseline demographic data as extracted from included studies is shown in Table 14.

Table 14 Population demographics of included studies

Study		Sample size	Avg Age Years	duration probiotics days	Gender M:F	Probiotic	overall	Probiotic group
		Total N Control/Inter				Compliance	Drop out	Disease duration Yrs.
Alipour et al 2014[218]	RA	N=60 (30/30)	41.14	56	0:60	67.6%	23.33%	5.25 (3.75,10)*
Brophy et al 2008	SpA	N=147(76/71)	44.8	84	45:31	67.9%	34.69%	20.3 (± 13,2)
Hattaka et al 2003[220]	RA	N=26 (13/13)	50	365	4:5	'good'	19.23%	8.3 (± 7.3)
Jenks et al 2010[221]	AS	N=63 (31/32)	45.5	84	19:13	92.5%	0.00%	9.8 (± 13)
Lee et al 2010[222]	RA	N=12	56	7	6:6	L. acidophilus. 83% S. salivarus 67% B. lactis 0%	0.00%	NS
Mandel et al 2010[223]	RA	N=45 (23/22)	62.9#	60	3:9	Not stated	2.22%	11.8 (± 5.4)
Pineda Mde L et al 2011[224]	RA	N=29 (14/15)	63.8	84	21:2	Not stated	10.34%	19 (± 12.4)
Shukla et al 2016[225]	SpA	N=46(23/23)	16*	84	23:21	98.1%	13.04%	3 (1.5,5)*
Tomasello et al 2015[226]	SpA	N=59 (28/31)	43.4	365	NS	NS	NS	NS
Zamani et al (2016)[228]	RA	N=54 (27/27)	49.3	56	22:3	>90%	7.41%	7.7 (± 6.1)
Zamani et al (2017)[229]Popula	RA	N=60 (30/30)	52.2	56	22:4	>90%	26.67%	7 (± 5.7)

*Median and IQR range provided # participant age not given for probiotic intervention alone

3.4.2 Condition

The impact of probiotics upon many types of inflammatory arthritis remains unexplored. Eight of the included studies focused on individuals with RA and the remaining four studies included a range SpA (including juvenile arthritis, ankylosing spondylitis and enteropathic arthritis) which are generically referred to throughout the results section as SpA.

3.4.3 Age

Immunosenescence may be a confounding factor affecting the impact of probiotic interventions in inflammatory conditions. As there were no significant differences in baseline age between intervention and control groups across studies data is being presented for the intervention group. Only Shukla et al.[225], was conducted with children, the median age of this study was 16 yrs. Excluding the paediatric trial data, the average age of participants (using a weighted average approach) receiving probiotics in trials concerning SpA was still younger (45 yrs.) than for those concerning RA (51 yrs.).

3.4.4 Disease duration

Inflammatory conditions have a long prodromal periods and distinct phases of the condition may present differerent capacity for modulation by probiotics.There was a large range of

disease duration described in the populations provided with probiotics. All samples achieved a mean duration of greater than three years indicating established, not early disease status. Three studies included participants with disease duration greater than 10 years. The average disease duration (calculated by weighted mean) of all recipients receiving probiotics was 11.7 years, however, when analysed by condition those with RA had a lower average disease duration of 9 years and those with any form of SpA an average of 14.5 years. Excluding the paediatric trial the average disease duration of those with a SpA form of arthritis in the study rose to 17.04 years.

3.4.5 Duration of intervention

The median duration of probiotic delivery across studies was 60 days (just over eight weeks). Only one study utilised a short time frame of seven days, and only one study used a long-time frame of a year. Therefore, the most frequently employed delivery time (mode) was 56 days (eight weeks). When reviewed by condition (RA versus SpA) a difference was identified. Studies utilising participants with RA had a shorter median duration of 56 days (8 weeks) compared to SpA median of 84 days (twelve weeks). When considering participant numbers (rather than trials), most individuals (93%) received probiotics for 56-84 days. Where intervention stated as three months without reference to days used, taken as standard 12-week intervention (84 days).

3.4.6 Gender

Gender and hormonal influences may affect the microbiome and response to probiotics. There was significance difference in gender balance in population samples across studies. One study population, discussed in two papers only used females.[218,227] Whereas in three studies the population was predominately males.[224,228,229] Not all studies described the gender balance of their sample groups.[226]

3.4.7 Patient adherence

Patient adherence is the level of compliance a patient has with taking their medication as prescribed. This should be considered alongside patient retention as both may affect the population size receiving the identified study intervention in the correct dose/ duration.

Most studies used tablet count or weight as a measure of compliance. Exceptions were self-report Brophy et al,[219] and faecal assay to assess probiotic levels in Lee et al.[222]

3.4.8 Patient retention

Patient retention may also be known as dropout. Average dropout rates across all clinical trials has been estimated at 30%.[232] Placebo-controlled trials may be more vulnerable to retention issues if individuals become concerned that their condition is not being well managed. Two included studies showed drop out above 30%. The highest overall drop-out was in the study by Brophy et al.[219,220] Drop out between intervention and control groups was similar in 7 out of the 10 studies that explicitly identified patient retention. Differential drop out with greater loss affecting intervention participants was seen in two studies, Hattaka et al.[220] (38% in intervention versus none in control) and Vaghref et al.[227] (26% in intervention versus 20% in control). Differential drop with greater loss of control participants was seen in two studies Pineda et al.[224] (21.43% in control versus none in intervention) and Shukla et al.[225](17% in control versus 8% in intervention).

3.4.9 Population exclusions

A wide variety of factors have been shown, or suggested, to affect the microbiota of the gut and alter the function of ingested probiotics and/or their capacity to colonise the gut. Therefore, the characteristics of participants that were excluded from the study trials were extracted. Whilst in clinical practice all factors may be considered by the medical practitioner before commencing a given form of intervention, this review employed a simple grouping of exclusion criteria aimed to clarify the transferability of outcomes to real world situations. The most commonly stated exclusions were current use of other supplements, recent use of antibiotics individuals with IBD, kidney disease and pregnant or breastfeeding women. The most commonly applied exclusion criteria were use of biologic disease modifying anti-rheumatic medications.

3.4.10 Probiotic formulations:

Organisms that may act as probiotics include viruses, fungi and bacteria. A simple outline of relevant classification was provided in Chapter 1, Figure 2 and 3. A key focus of this study is to identify any change in outcome with respect to specific probiotics supplied, therefore a detailed of table of probiotic inclusions is provided below in Table 15.

Table 15 Probiotic formulation per included study in colony forming unites (CFU)	Alipore B, et al	Brophy S, et al.	Hatakka K, et al.	Jenks K, et al	Lee HJ, et al	Mandel DR, et al.	Pineda Mde L, et	Shukla A, et al.	Tomasello G, et al.	Zamani B, et al 2016	Zamani B, et al 2017	Vaghef-Mehraban
S. boulardii									X			
B. lactis				4	4							
B. breve								X				
B. Longum								X				
B. infantis		12.5						X				
B. bifidum		12.5								20	20	
L.acidophilus				4	4				X	20	20	
L. salivarius		62.5							X			
L. rhamnosus			50				10					
L. plantarum								X				
L.paracasei		12.5						X				
L. delbrueckii								X				
L.caseii	1									20	20	1
L.reuterii							10					
Strep. thermophilus								X				
Strep. salivarus				1	1							
Enterococcus faecium												
Bacillus coagulans						20						
Taken daily	once	once	2x twice	twice	twice	once	twice	twice	twice	once	once	
TOTAL CFU in 24 hrs 10^8	1	100	200	18	18	20	40	2250		60	60	

Probiotics species

From the 12 included studies only one study, involving 31 participants utilised a yeast (*Saccharomyces boullardii*) and the rest of the studies employed bacteria. The specific probiotic species and the concentration in which it was supplied, where known, has been provided for each study in Table 15 above. The studies included a variety of bacterial organisms, classified within three different orders. There were 17 different bacteria species delivered with the formula of the 12 studies. The numbers of individual participants receiving specific species are represented in Figure 8 below and have been colour coded to identify their shared phylum. The order and genus of bacteria delivered to the most participants across the studies was that of Lactobacillus.

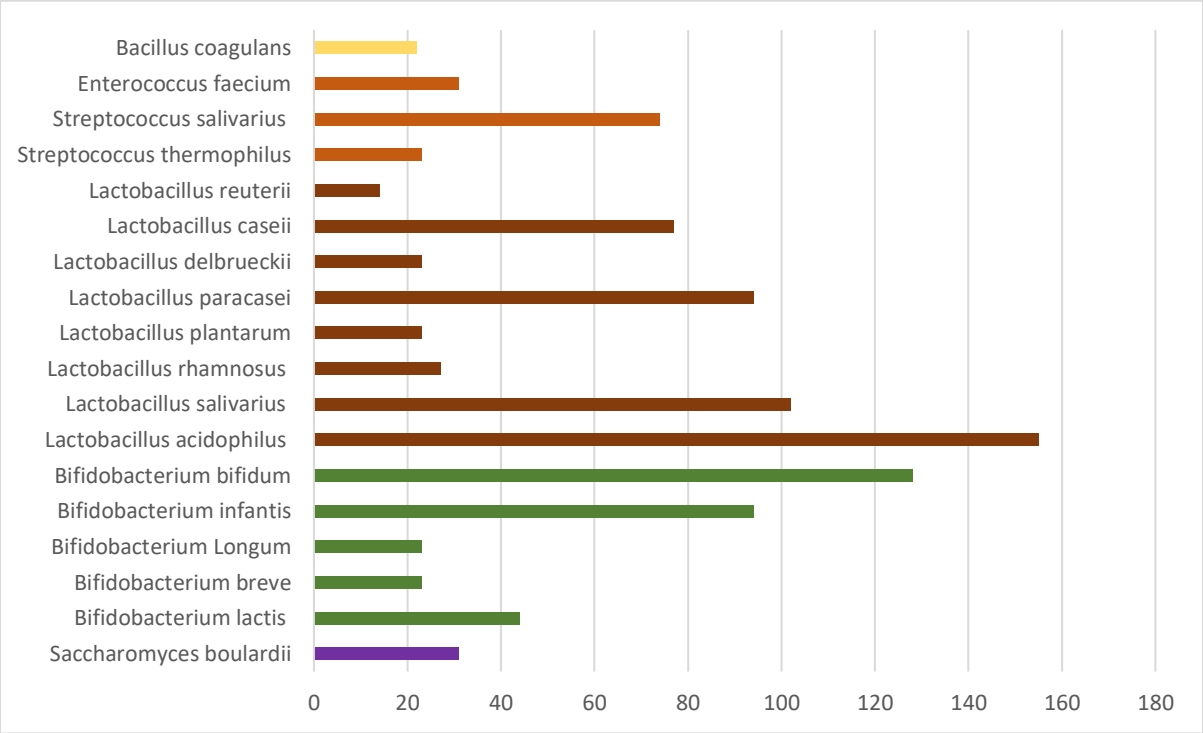


Figure 8 Total number of participants receiving each species of probiotic.

Probiotic combinations

The formulation and number of bacterial species within the probiotic intervention was varied, from those containing single species to formulations with up to eight different species. A combination of three or four types of probiotic was commonly employed and given to the majority number of participants. Only one study by Zamani et al.[229] included specific prebiotics in the form of 800mg of inulin.

Probiotic concentrations

Concentration of a probiotic formula is measured in colony forming units. For comparability data is taken as the CFU in 10^8. Probiotic formulations were delivered heterogeneously across studies, in single daily or twice daily doses. Therefore, the actual amount of the probiotic received per 24 hours was calculated. As demonstrated in Figure 9 Shukla et al.[225] provided the greatest concentration of probiotics to participants. Alipour et al.[218] (and therefore Vaghref-Mehrabany et al.[227]) supplied the lowest concentration. Due to duplication of study participants and intervention, any graphs or tables relating to duplicated information display that related to Alipour et al.[222]

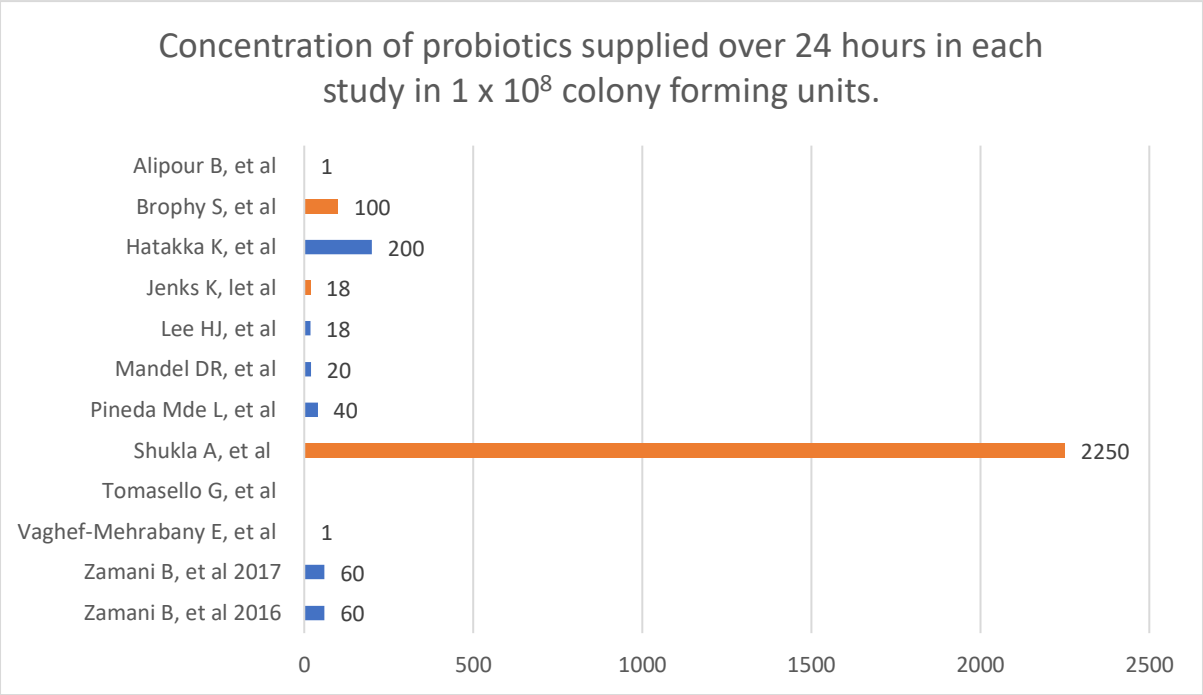


Figure 9 Total concentration measured in Colony Forming Units (CFU) of probiotics supplied in 24 hours by each study.(RA represented in Blue, SpA represented in orange)

3.5 Outcomes

3.5.1 Life Impact

Quality of life and patient reported well-being

Five studies employed patient reported wellbeing scores on a visual analogue scale from 0-10. Details are provided in Table 16. Two studies also assessed a broader quality of life measure the health assessment questionnaire. Heterogeneity of study participants was apparent with Brophy et al.[219] having a significantly longer disease duration, Jenks et al.[221] having the only study with a predominately male population and Pineda et al.[224] having the

oldest population. Only one study employed a high concentration formula.[219] Two studies employed single species formulation, *Lactobacillus caseii* in Alipour et al.[218] and *Bacillus coagulans* in Mandel et al.[223] All studies except Mandel et al. [223] incorporated probiotics within the Lactobacillus family. Effect scores calculated by the authors indicated a statistically significant trend towards improvement in quality of life. When post test scores were analysed to create a standardised mean difference a statistically significant benefit of probiotic administration was found, effect size -0.37 (95%CI -0.59,-0.15) see Figure 10. Data conversion was used as per the methodology protocol and as decribed in the Cochrane handbook.[216] The application of an average standard deviation may underlie the different findings between original authors and this review.

Table 16 Outcome of administered probiotic in included studies on patient wellbeing as measured with a variety of scoring systems.

Study	Measure	Probiotic Mean Post-test and SD	Placebo Mean Post-test and SD	SMD and 95% CI
Alipour et al 2014[218]	Global Health Score	20.92 (±27.94)	35 (±40.98)	-0.40 (-0.91,0.11)
Brophy et al 2008[219]	BAS-G	2.9 (±2.3)	3.7(±3.3)	-0.28 (-0.64, 0.05)
Jenks et al 2010[221]	PtGa wellbeing	2.7(±2)	3.3 (±2.4)	-0.27 (-0.76,0.22)
Mandel et al 2010[223]	PtGa			Raw Data unavailable
Pineda Mde L et al 2011[224]	PtGa Disease activity	2.71 (±1.75)*	4.69 (±1.75)*	-1.1 (-1.88,-0.32)
Hattaka et al 2003[220]	HAQ	0.5 (±0.4)	0.7 (±0.7)	-0.34 (-1.11, 0.43)
Pineda Mde L et al 2011[224]	HAQ			n/a

*Data conversion applied CI (confidence interval) SMD (standardised mean difference)
BAS-G(Bath Ankylosing Spondylitis Patient General score) (Patient Global Assessment) HAQ (Health Assessment Questionnaire) PtGa

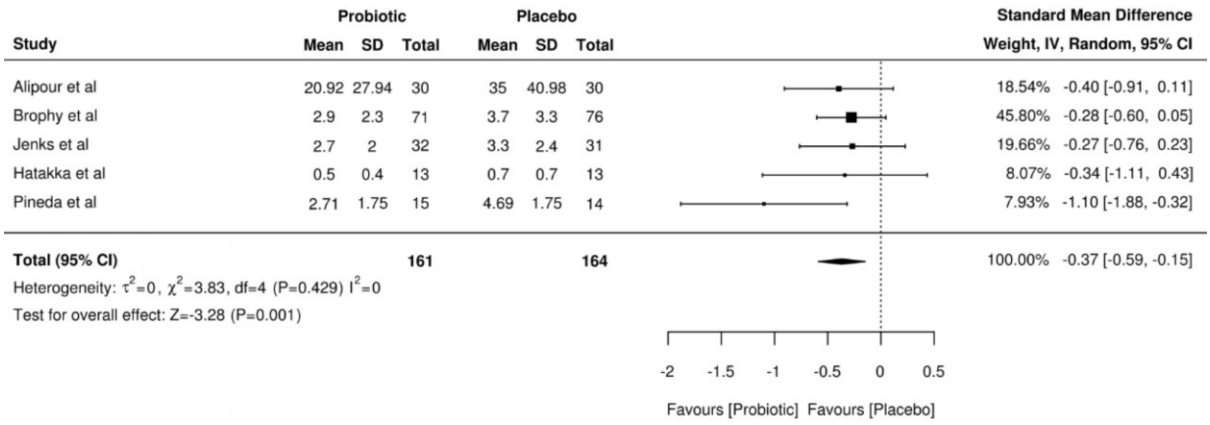


Figure 10 Forest plot displaying the meta-analysis of five individual studies of probiotic effect upon patient reported general wellbeing and quality of life, measure with patient general assessment (Pt GA) and Health assessment questionnaire (HAQ).

A sensitivity analysis was undertaken, see Figure 11, by removing the study where data was converted.[224] A random model was used as whilst the number of studies investigated was below the threshold of five recommended for random effects models by the JBI reviewers handbook, it enables a comparable forest plot to examine the effect of leaving one study out of the meta-analysis. The result remained a positive statistical benefit of probiotics upon patient wellbeing. p=0.009.

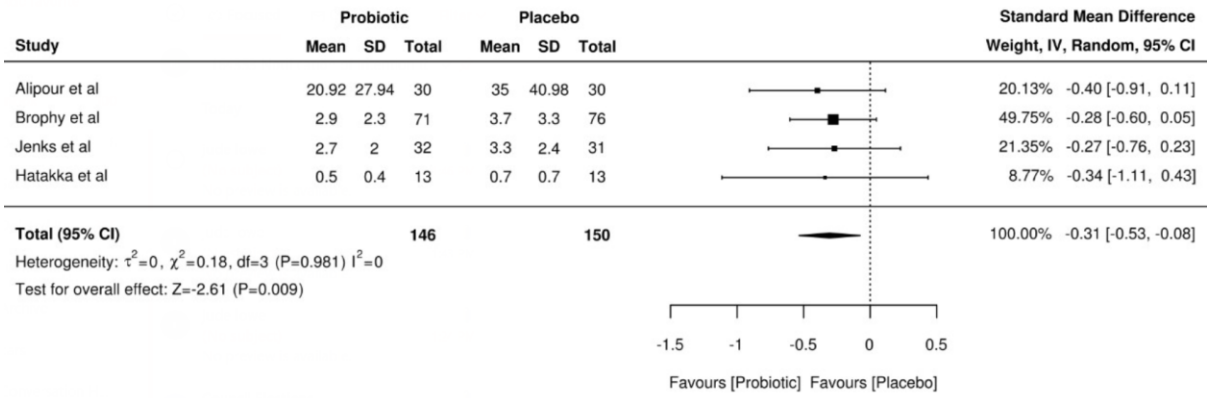
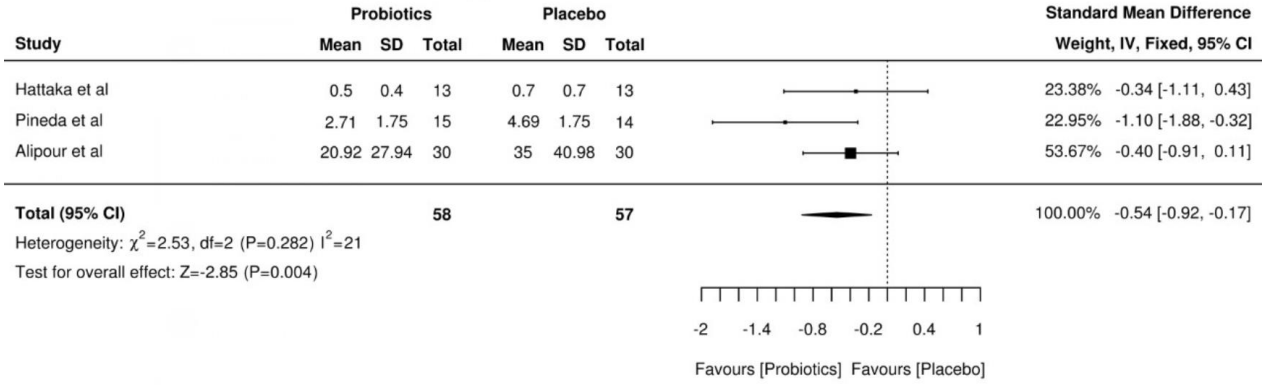


Figure 11 Forest plot displaying sensitivity analysis for 4 individual studies of probiotic effect upon patient reported general wellbeing and quality of life.

Subgroup analysis was conducted to investigate the specific effect of different probiotic formulation. Studies that employed only Lactobacillus forms of probiotic were compared to those that contained both Lactobacillus and Bifidobacterium genera.

The subgroup analysis as shown in Figure 12 a and b, revealed a stronger effect from the use of Lactobacillus compared to combination Lactobacillus and Bifidobacterium products. Identifying further differences from the Lactobacillus and Bifidobacterium mixtures relevant to specific species is not possible. However, within the Lactobacillus only sub-group there did appear to be differences within formulations. For example, formulations containing *Lactobacillus rhamnosus* had greater effects size than *Lactobacillus caseii* alone. It should be noted that confounding effects of differing concentrations of formulation may be relevant as the purely *Lactobacillus caseii* formulation also had the lowest concentration.

A) Lactobacillus



B) Lactobacillus and Bifidobacterium

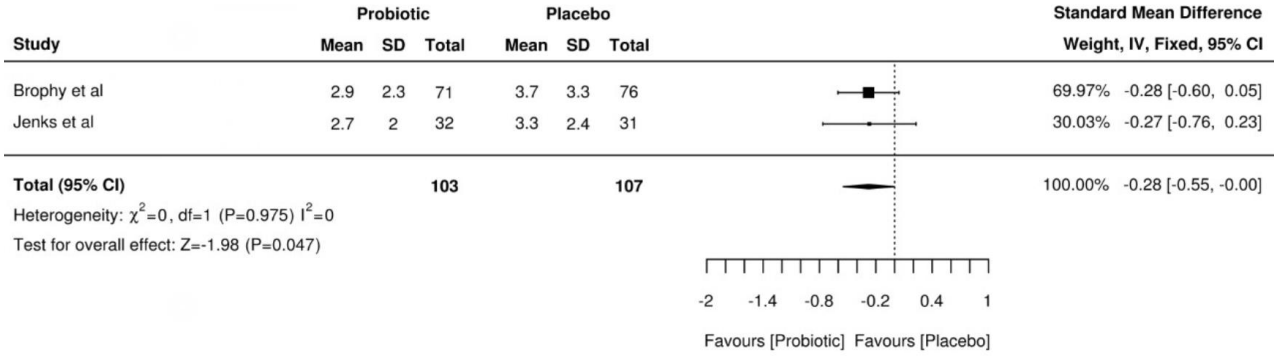


Figure 12a & b Forest plot displaying subgroup analysis of two individual studies sub-grouped by probiotic formulation upon Patient reported general wellbeing and quality of life

Fatigue

Two studies measured fatigue after a 12 week probiotic intervention, Pineda et al.[224] utilised a visual analogue scale to score fatigue on a uni-dimensional scale with RA patients and Jenks et al.[221] employed a fully validated multidimensional tool with SpA patients. Results are shown below in Table 17. Whilst both studies provided a relatively low once daily dose of probiotics, the formulation of probiotic varied. Pineda et al.[224] used a combination of two probiotics within the Lactobacillus family whereas Jenks et al.[221] used a combination of three probiotics, two Lactobacillus and one Streptococcus species however all three remain with the broader Lactobacillus genera.

Significant heterogeneity in sample population demographics was seen between studies. The trial population was predominately female (93%) older (mean age 63 yrs.) and long disease duration (mean 19 years ± 12.4 years) in the study by Pineda et al.[224] Jenks et al. [221]

included a predominately male population of younger age (mean 45.5 years) and shorter disease duration (mean duration 9.8 yrs.). The probiotic was supplied once daily in a dose of 2x 10^9 colony forming units per strain. In both studies baseline fatigue levels were greater than for established healthy population norms but below the norms established for individuals with RA or SpA respectively. [140,233] Mean clinically important differences were not achieved in either study.

Table 17 Outcome of administered probiotics on fatigue measured on two different scales, Visual Analogue Scale for fatigue (VAS-f) and the multi-dimensional assessment of Fatigue Scale (MAFS).

Study	Measure at 12 weeks	Probiotic Post-test mean and SD	Control Post-test mean and SD	SMD and 95% CI
Jenks et al[221]	MAFS	21.9 (±10.2)	23.9 (±11.1)	-0.19 (-0.68,0.31)
Pineda et al[224]	VAS	2.88 (±2.00)	5.7 (±2.00)	-1.73 (-2.18,-0.56)

* Calculated using mean changes in patients from baseline to final visit CI (confidence interval) SMD (standardised mean difference) MAFS (Multidimensional Assessment of Fatigue Scale) VAS (Visual Analogue Scale)

Using identical method on both data, post-test effects size was calculated, and a forest plot generated as shown in Figure 13. When calculating effects size from post test scores alone, a small benefit for the administration of probiotics was seen for both studies and whilst combination suggests a statistically significant effect when combined the fixed effects forest plot revealed a very high level of heterogeneity (I^2=83) indicating that it is inappropriate to combine the results of these two studies and that further data is required to assess the effect of probiotics upon fatigue.

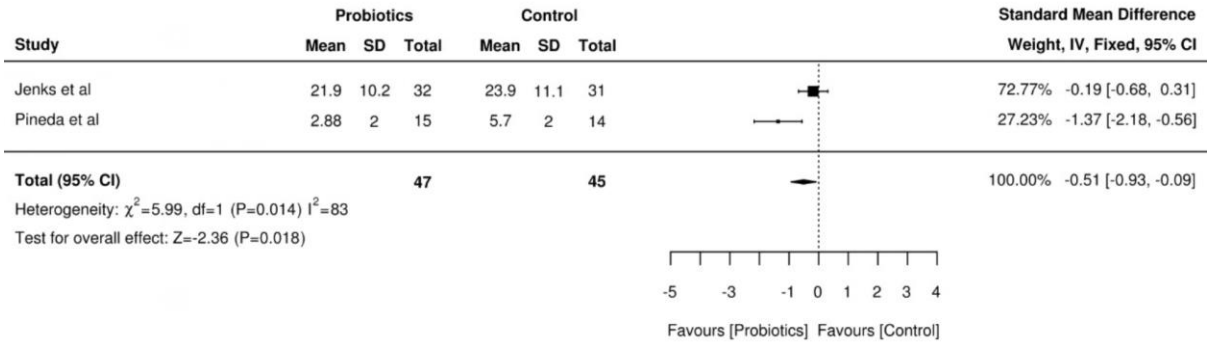


Figure 13 Forest plot representing the meta-analysis of two studies investigating the effect of probiotics upon fatigue measured by Visual Analogue Scale (VAS) and Multidimensional Assessment of Fatigue Scale(MAF).

Bowel symptoms

Two studies measured change in bowel symptoms after a 12-week probiotic intervention for individuals with SpA. Brophy et al.[223] utilised a portion of the visual analogue scale for Irritable bowel syndrome (including diarrhoea, stomach pain and blood in stool) on a scale of 0 to10 whereas Jenks et al.[225] used a fully validated multidimensional tool the Dudley Inflammatory Bowel symptom questionnaire. On both scales a reduction in score is seen as an improvement in symptoms.

Patient demographics were similar in each group being predominately male of similar age, however the disease duration was significantly longer in the study by Brophy et al. [223] Results are shown below in Table 26.

Brophy et al.[223] provided a combination of four different probiotics, two Lactobacillus and two Bifidobacterium , supplying a total of 10 x 10^9 colony forming units per 24 hrs. Jenks et al. [225] provided a lower concentration product 9 x 10 [8] containing three different families of probiotic (*Bifidobacterium lactis, Streptococcus salivarius* and *Lactobacillus acidophilus*) but supplied twice a day, therefore providing a total of 1.8 x 10^9 over 24 hours. Baseline bowel symptoms varied between studies. Whilst both had symptoms above healthy baseline norms the baseline bowels symptom levels as measured with the Dudley questionnaire were below the threshold at which symptoms should affect quality of life. The authors outcomes are provided in Table 18 and neither identified a statistically or clinically significant benefit from probiotic administration.

Table 18. Outcome of administered probiotics in included studies on Bowel symptoms

Study	measure	Probiotic at 12 weeks Post-test mean and SD	Placebo at 12 weeks Post-test mean and SD	SMD and 95% CI
Brophy et al [223]	VAS (IBS)	1.4 (±2.3)	1.0 (±1.7)	0.48 (0.16,0.81)
Jenks et al[225]	DISQ	6.6 (±5.1)	8.8 (±8.0)	-0.33 (-0.82,0.16)

CI (confidence interval) DISQ (Dudley Inflammatory Bowel Symptom Questionnaire) VAS (Visual Analogue Scale) IBS (Irritable Bowel Syndrome) SMD (standardised mean difference)

When calculating effects size from post-test scores alone, no benefit for the administration of probiotics was seen, effect size 0.04, which would not be considered statistically significant (95%CI -0.23 to 0.31). When calculating effects size from post-test scores without adjustment the same conclusion was reached as seen in Figure 14. A fixed model for meta-

analysis was employed as the number of studies investigated was below the threshold of five recommended for random effects models by the JBI reviewers handbook. As both study lines cross the line of null effect, the plot does not illustrate a statistically significant result and high heterogeneity (I^2=66) indicates it is inappropriate to combine these results, and further studies are required.

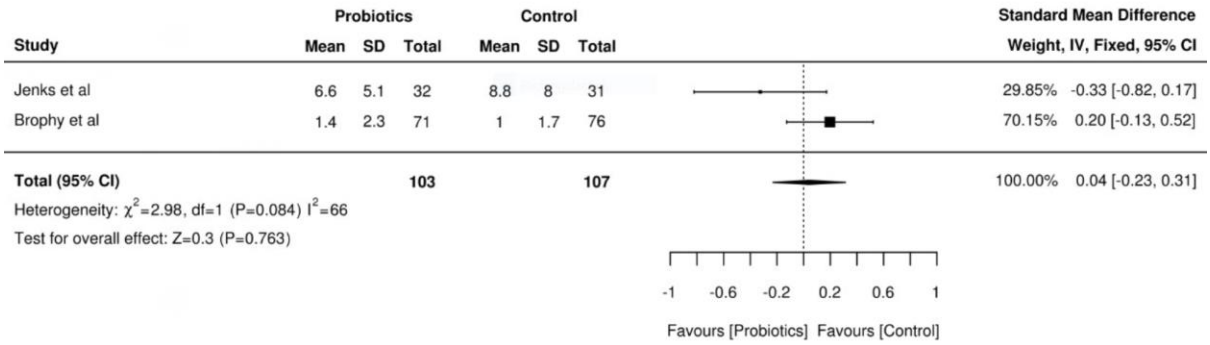


Figure 14 Forest plot displaying the meta-analysis of two studies investigating probiotics on bowel symptoms.

Pain

Data from five studies provided direct outcomes of pain as measured with a visual analogue scale, see Table 19. Two studies individually concluded no significant difference in pain scores after the application of mixed formula probiotics in low concentration for patients with SpA and RA.[221,224] Three further studies using change from baseline, identified a statistically significant reduction in pain in the intervention groups. These studies included two with a high concentration multi-formula probiotic (Zamani et al.[228,229]) and one with a single species low concentration formula (Vaghef-Mehrabany et al.[227]). All studies which identified probiotic benefits were conducted on individuals with RA. Heterogeneity in visual analogue scale occurred so all results were converted to the 100 point scale as recommended by Busse et al.[234] and mean difference was then employed.

Table 19 Outcome of probiotic administration on pain using Visual Analogue Scale (VAS) as outcome.

Study	Probiotic VAS 0-100		Control VAS 0-100		
	Mean (SD)	% change from baseline	Post Mean, SD	% Change from baseline	Effect size SMD 95% CI
Jenks et al[221]	27 (± 25)	-6.90%	26 (±22)	-13.33%	1.00 (-10.62,12.62)
Pineda Mde L et al[224]	34.8 (±21.5)	-6.76%	52.2 (±21.5)	0.2	-17.7(-33.36,-2.04)
Zamani et al[228]	22 (±27)	-52.96%	38 (±26.2)	-24.26%	-8.90 (-20.60,2.80)
Zamani et al[229]	27(± 15.6)	-43.15%	35.9 (±26.8)	-23.54%	-16.0 (-29.46,-2.54)
Vaghef-Mehrabany et al[227]		-43.90%		-5.99%	N/A

*CI (confidence interval) SMD (standardised mean difference)

When re analysed using post-test statistical analysis the effects size, as shown in Figure 15. indicates a small positive and statistically significant effect of probiotic administration on patient reported pain in RA. Fixed model analysis was employed due to the low number of studies and heterogeneity was moderately high but below the 50% threshold for considering meta-analysis suitable.

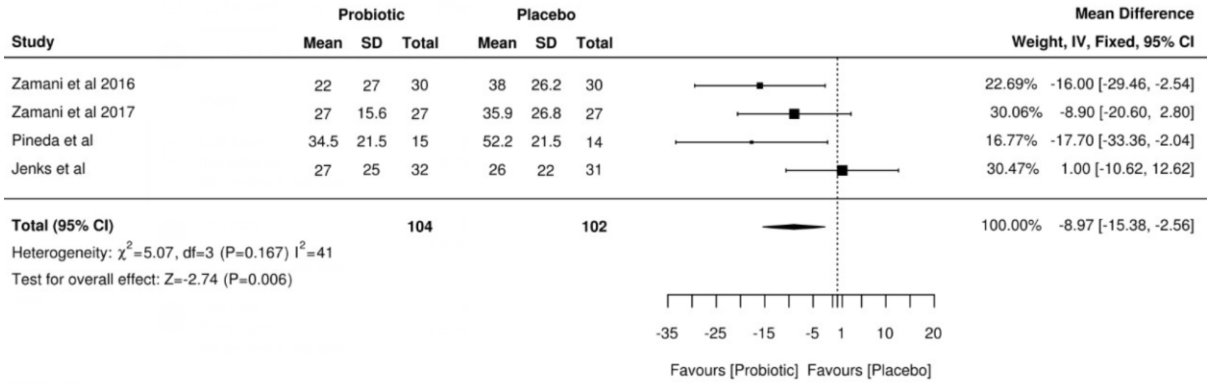


Figure 15 Forest plot displaying the meta-analysis of four studies investigating effect of probiotics upon patient reported pain using Visual Analogue Scale (VAS)

A minimal clinically important difference for the visual analogue scale is often stated as 1.0 (10 on a 100 point scale), in which case this was only achieved in two studies. It been suggested that this may not be meaningful to patients and that the greater difference of 20 point change on a 100 scale is employed.[235] Using this alternative approach would suggest that there was no clinically meaningful change in pain scores in any of the studies included.[234] Assessing the impact of pain on individuals is not simple and it is known that the starting value may affects the magnitude of the change from a patients perspective. An alternative approach has been suggested to use the percentage change from baseline. Using this approach, higher changes as a percentage from baseline were reported in the groups receiving probiotics and therefore it cannot be assumed that patients will not gain important benefits from this intervention.

As only one study employed a purely Lactobacillus formulation, a sensitivity analysis was performed to identify if any change in effect size was identified when only studies employing a mix of Lactobacillus and Bifidobacterium formulations were included in the meta-analysis. The sensitivity analysis is shown in Figure 16.

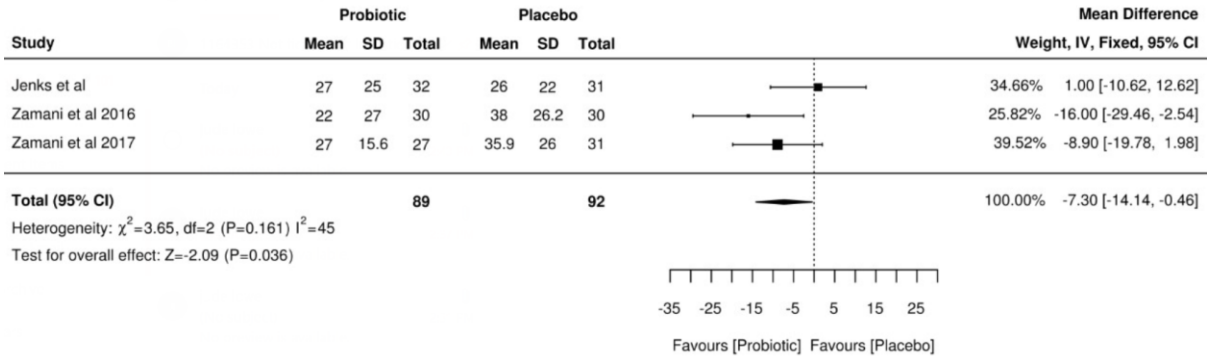


Figure 16 Forest plot displaying the meta-analysis of three studies investigating effect of mixed probiotics formulations upon patient reported pain (VAS).

The removal of the only study employing purely Lactobaccillus reduced the size of the effect score, but it remained. statistically significant. Whilst visual analogue pain scores were only reported by five studies, tender joint scores were reported by eight studies therefore this was mined as an alternative pain outcome measure as shown in Table 20.

Table 20 Outcome of probiotic on tender joint score.

Study	Probiotic outcome	Control outcome	Effect size, Mean Difference and 95%CI (post test scores)
Hatakka et al[220]	2.5 (±1.7)	2.6 (± 2.4)	-0.05 (-0.82,0.72)
Jenks et al[221]	3.1 (± 3.9)	5.4 (± 8.8)	-0.34 (-0.83,0.16)
Mandel et al[223]	Raw Data Not available		N/A
Pineda Mde L et al[224]	12.87 (11.89)	-8.97 (4.4)	0.55 (-0.19,1.30)
Shukla et al[225]	2.33 (± 2.175)	0.5 (± 2.175)	0.83 (0.22,1.43)
Zamani et al[228]	4.8 (± 2.2)	4.7 (± 2.4)	0.04 (-0.46,0.55)

BAS-G(Bath Ankylosing Spondylitis Patient General score) HAQ (Health Assessment Questionnaire)

A meta-analysis, as shown in Figure 17, was conducted with five studies where raw tender joint score data was available. No significant benefit of probiotics upon tender joint scores was found. Formal joint counts have been described with the evaluation of anywhere between 28 to 80 joints, as the specific number of joints assessed was not detailed in all studies standardise mean difference was used for the met analysis. Data presented as median and interquartile range was converted as detailed in Chapter 2.

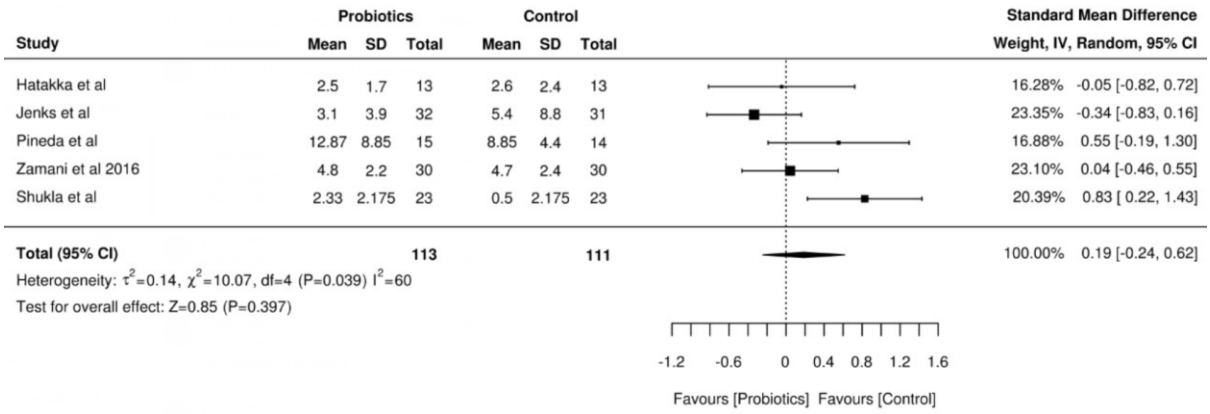


Figure 17 Forest plot for the meta -analysis of probiotic administration on tender joint score

3.5.2 Adverse events

Adverse effects were reported in 11 of the 12 studies included in this review are displayed below in Table 21.

Table 21 Outcome of administered probiotics on adverse effects.

Study	Probiotic			Control			RR and CI	P	NNT harm
	Pop	Adverse effect		Pop	Adverse effect		ANY event		
	N=	Major	Minor	N=	Major	Minor			
Alipour et al[218]	30	0	0	30	0	0	-		
Brophy et al[219]	76	0	6	71	0	5	**1.12 (0.36,3.51)**	**0.86**	**135**
Hatakka et al[220]	13	0	0	13	0	0	-		
Jenks et al[221]	32	0	14	31	0	12	**1.09 (1.56,2.08)**	**0.8**	**40**
Lee et al[222]	12	0	4	-	-	-	-		
Mandel et al[223]	22	0	4	23	0	3	**1.33 (0.33,5.37)**	**0.69**	**26**
Pineda Mde L et al[224]	15	0	0	15	0	0	-		
Shukla et al[225]	23	1	9	23	1	11	**0.88 (0.44,1.76)**	**0.73**	**25**
Tomasello et al[226]	28	0	0	31	0	0	-		
Zamani et al[228]	30	0	0	30	0	0	-		
Zamani et al[229]	27	0	0	27	0	0	-		

CI (confidence interval) RR (relative risk) NNT (number needed to treat)

Only Tomasello et al.[226] did not state any information regarding side effects or adverse events in their study. Overall there was a lack of clarity regarding the definitions of mild/minor as opposed to serious adverse events. If including studies where adverse effects were not reported, and those where zero occurrences were reported, 50% of studies did not identify any effects from the administration of probiotics. This is reassuring but may also indicate a lack of standardised reporting in place. A forest plot of the meta-analysis of

relative risk of side effects is provided in Figure 18. No increase risk as a result of taking probiotics was identified, relative risk 1.02.

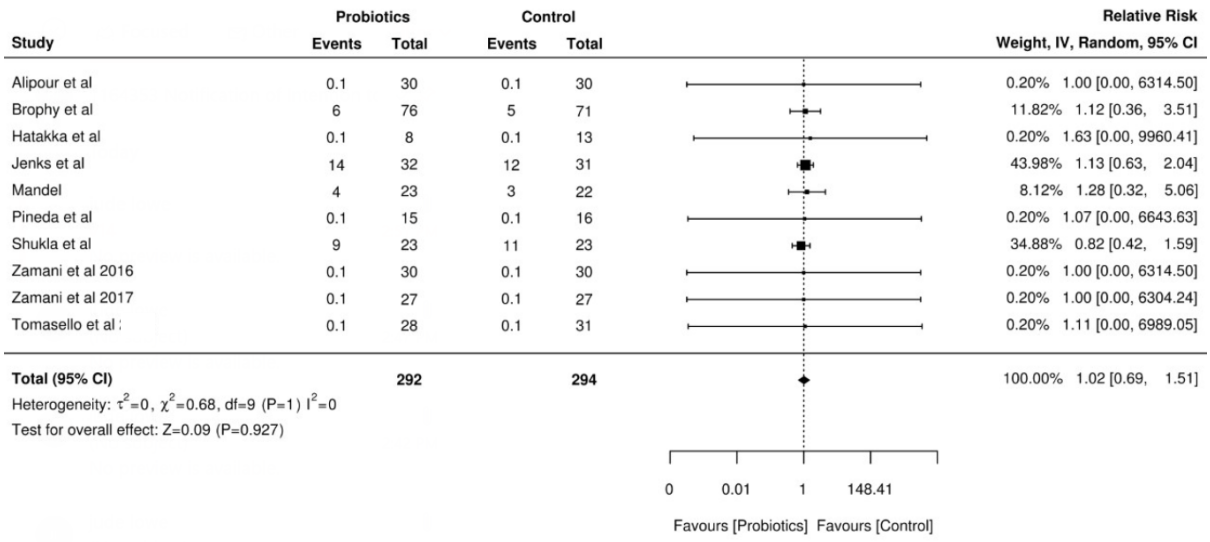


Figure 18 Forest plot representing the meta-analysis of 9 studies investigating the relative risk of minor adverse effects after the use of probiotics.

Table 22 places these reported effects into the Common Terminology Criteria for Adverse Events and identifies equivalent rates of adverse effects between intervention and control/placebo groups.

Table 22. Common Terminology Criteria for Adverse Events (CTAE) categorisation of reported adverse effects by included studies.

Classification	Symptom severity	Modification of treatment	Total probiotics	Total control
Grade 1	Mild/Asymptomatic	Observation only	16% (33/211)	15% (31/206)
Grade 2	Moderate	Minimal non-invasive intervention or limitations of ADL	0.5% (1/211)	0.5% (1/206)
Grade 3	Severe	Requires hospitalisation and disabling	0	0
Grade 4	Life threatening	Urgent intervention required	0	0
Grade 5	Death		0	0

Only Shukla et al.[225] reported any serious adverse events. It should be noted that the serious side effects experienced were due to severe diarrhea in the placebo group and pulmonary tuberculosis in the probiotic group, with neither likely attributable to the intervention. The risk of a minor adverse effect from each study, calculated taking an intention to treat

approach, varied from 0.88 to 1.33 which indicates approximately the same number of events being reported by individuals in the control group as in the intervention group.

Zero event trials were included in the meta-analysis as recommended.[236] The study by Lee et al.[222] did not include a control group and administration of probiotics in the intervention group resulted in 33% of participants experiencing a minor adverse event. A trend, as shown in Figure 19, was observed that more studies on individuals with SpA reported adverse effects. However, subpopulation analysis applied to the studies to compare SpA versus RA did not find any statistical difference between outcome.

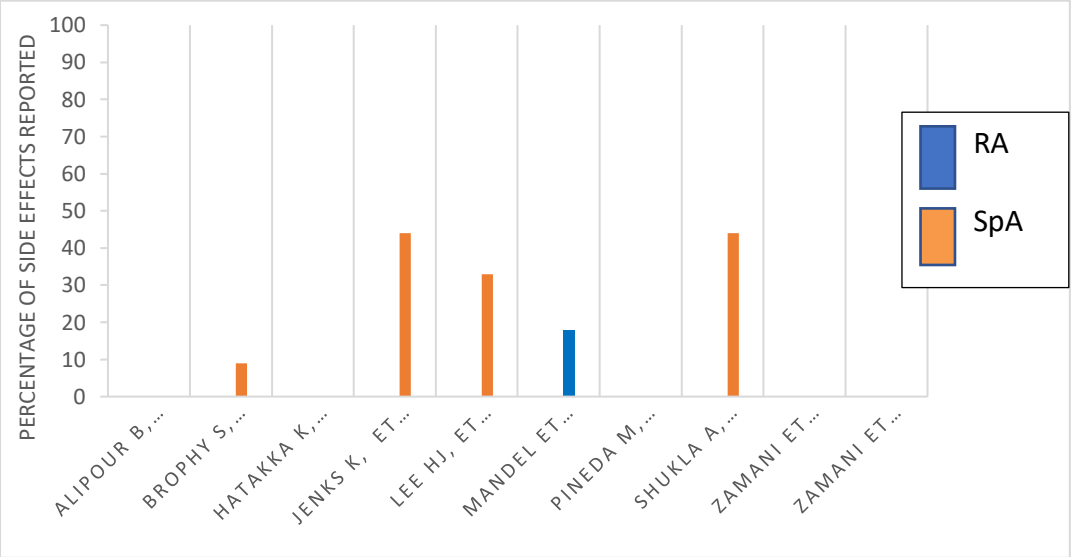


Figure 19 Percentage of individuals that reported side effects in intervention groups across included studies.

Data from two studies provided a more detailed breakdown of specific types of adverse events. Increased flatulence was the most common adverse events which gave a relative risk of 2.03 (95%CI 0.45,9.5 p=0.36 Shukla et al.[225]) and 2.03 (95%CI 0.57,7.25 p=0.27 Jenks et al.[221]). Whilst this result was not statistically significant, and flatulence is a minor side effect it indicates a Number needed to treat (harm) of ten for both studies. It should be noted that there was no use of validated adverse events scales and that many relied on end point self-report to investigators. A wide range of other minor adverse effects were also noted with nausea the next most commonly reported symptom, the authors indicated that concurrent anti-rheumatic medications were likely causes for most nausea cases.

3.5.3 Markers of systemic inflammation

C-Reactive Protein

Nine studies included C-reactive protein measured in mg/L as an outcome of interest. Despite significant heterogeneity in population demographics, probiotic interventions and formulations all studies indicated a negative effect size, indicating a possible reduction in inflammation due to the intervention of probiotics. Access to full data enabled seven of these studies to be used in a meta-analysis. The confidence interval crossed the midline in all studies except Zamani et al.[229] as shown in Table 23.

Table 23 . Outcome of administered probiotics for included studies on C-Reactive Protein (CRP) in mg/L

Study	Probiotic Post-test and SD	Control Post-test and SD	MD CRP mg/L 95% CI
Alipour et al[218]	2.8 (±4.79)	3.5 (±7.43)	-0.70 (-3.86,2.46)
Hatakka et al[220]	2.6 (± 3.3)	7.4 (±8.7)	-4.80 (-9.86,0.26)
Jenks et al[221]	6.7 (± 6.3)	11.3 (±11.2)	-4.6 (-9.11,-0.09)
Lee et al[222]	9.5 (±9.9)	N/A	N/A
Mandel et al[223]	Data Not avail	Data not available	N/A
Pineda Mde L et al[224]	5.6 (± 6)*	8.6 (± 6)*	-3.00 (-7.37,1.37)
Shukla et al[225]	2.96 (± 4.39)*	1.5 (±4.39)*	1.46 (-1.23,4.15)
Zamani et al[228]	6.61(±6.03)	9.09 (±7.46)	-2.48 (-5.91,0.95)
Zamani et al[229]	4.61(±2.71)	8.47 (±6.83)	-4.39 (-7.12,-1.66)

* Data conversion applied ^ standardised AUC values analysed. CI (confidence interval) CRP (C-reactive Protein) MD (mean difference) SD (standard deviation)

A meta-analysis revealed a statistically significant benefit of probiotics on C-reactive protein, see Figure 20. The size and significance of this effect may reasonably be expected to increase if the additional data from the other positive studies was included.

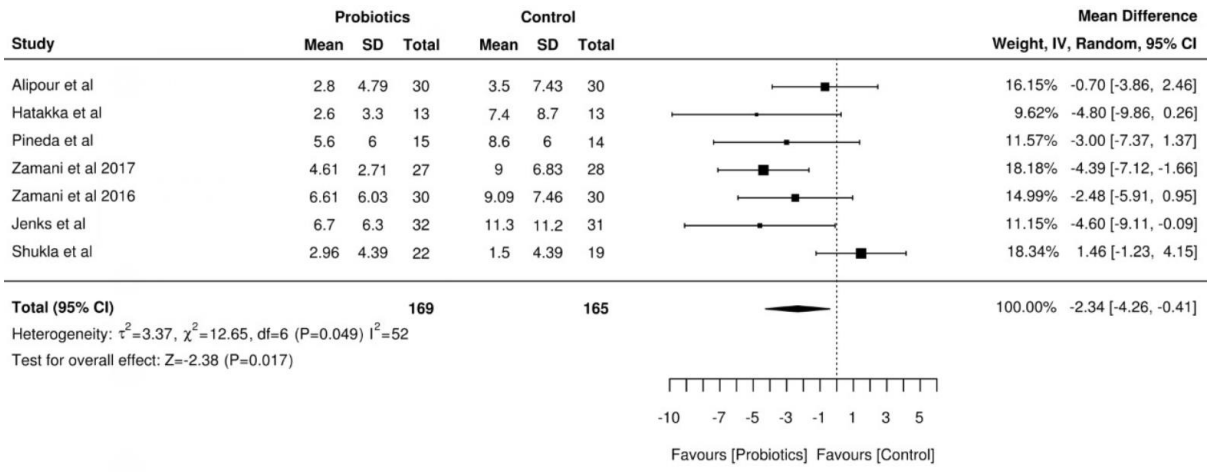
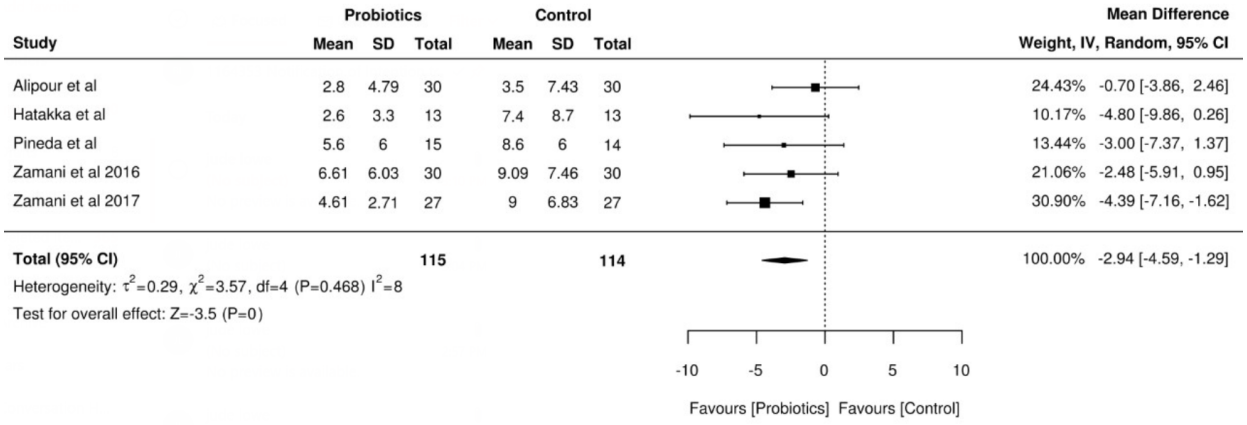


Figure 20 Forest plot representing the meta-analysis of seven studies investigating the effects of probiotics on C-Reactive Protein (CRP).mg/L

As prior reviews have consistently concluded that probiotic intervention can lower C-reactive protein in individuals with RA, a subpopulation analysis was performed as shown in Figure 21a,b. This aimed to identify population specific effects between individuals living with SpA compared to RA.

A) Rheumatoid Arthritis patient demographic only



B) Spondyloarthritis patient demographic only

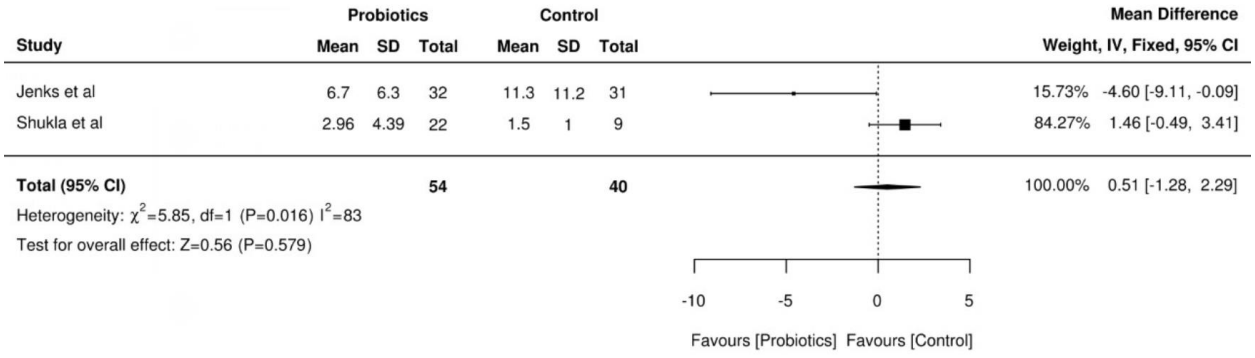
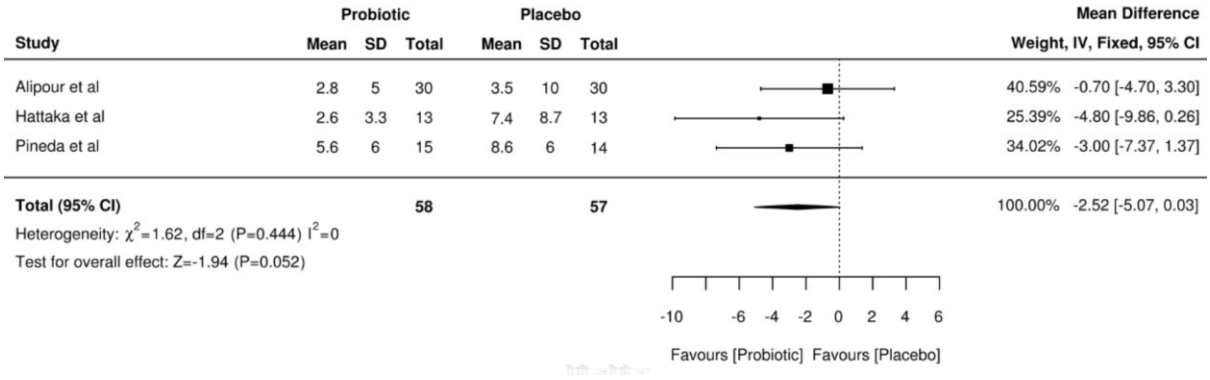


Figure 21a and b. Forest plot representing the sub population meta-analysis of 5 studies investigating the effects of probiotics on C-Reactive Protein (CRP mg/L) on individuals with rheumatoid arthritis(RA) compared to Spondyloarthritis (SpA)

Looking at the sub population of SpA did not reveal a benefit of probiotics on C-reactive protein, and there was high heterogeneity as shown by I^2=83 indicating that meta-analysis not appropriate for these studies.

A sub-population analysis was performed as shown in Figure 22 a and b. This aimed to identify formulation specific effects between probiotic that include both genera and those that used only Lactobacillus.

A) Lactobacillus



B) Lactobacillus and Bifidobacterium

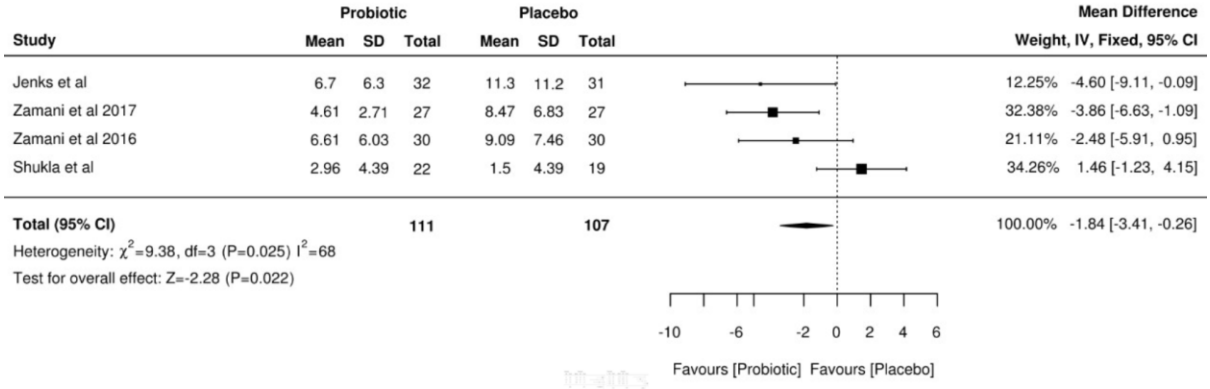


Figure 22a and b. Forest plot representing the sub population meta-analysis of 7 studies investigating the effects of probiotics on C-Reactive Protein (CRP mg/L) on individuals according to probiotic formulation.

Individual study results indicate a trend in favour of the ability of probiotics to reduce C-reactive protein, and when combines in a meta-analysis a statistically significant effect size is observed with low heterogeneity. Sub population analysis revealed a statistically significant decrease was only seen in studies for individuals with RA.

Sub population analysis targeting studies which provided different formulations of probiotics revealed a statistically significant benefit was only obtained by the mixture of probiotics (Lactobacillus and Bifidobacterium) compared with Lactobacillus alone. It should be noted that raw data from three studies which describe statistically significant reductions in c- reactive protein after the application of probiotics (two based on patients with RA and one based on patients with SpA) were not available for the meta-analysis raw data but would add to the effect size described.

Composite disease activity score DAS28

Four studies provided available data on DAS28 for a meta-analysis as detailed in Table 24. Whilst a further study by Hatakka et al.[220] provided data on the composite elements, it was not statistically appropriate to reconstruct mean DAS28. The study by Lee et al.[222] provided only baseline DAS28 data. All studies concerned patients with RA and similar demographics. All indicated a beneficial outcome for groups receiving active probiotic intervention. Meta-analysis, as shown in the forest plot in Figure 23, revealed a statistically significant benefit (effect size -0.28) of probiotics on disease activity score p=0.016.

Clinically significant reductions in disease activity score using C-reactive protein, are estimated as a one point drop overall, therefore the combined effects size did not reach clinical significance. Moving between accepted disease categorisation groups may also be meaningful with respect to change in medical management. None of the studies included individual with high baseline scores, but drops from moderate to low disease state were seen in Zamani et al. [229] and sufficient to meet remission criteria occurred in Pineda et al. [224]

Table 24 Outcome of administered probiotic in included studies on Disease Activity Score (DAS28)

Study	Disease Activity Score (DAS28)		
	Probiotic outcome	Control outcome	SMD and CI
Alipour et al[218]	2.07 (±0.82)	2.23 (±0.86)	-0.16 CI(-0.59,0.27)
Pineda Mde L et al[224]	2.08 (± 0.98)	1.93 (±0.98)	0.15 CI(-0.56,0.86)
Zamani et al[228]	3.7 (± 0.7)	4 (± 0.7)	-0.30 CI(-0.65,0.05)
Zamani et al[229]	2.6 (± 0.7)	3.2 (±1.1)	-0.60 CI(-1.09,-0.11)

**p value for between group differences final to baseline. CI (confidence interval) SMD (standardised mean difference)

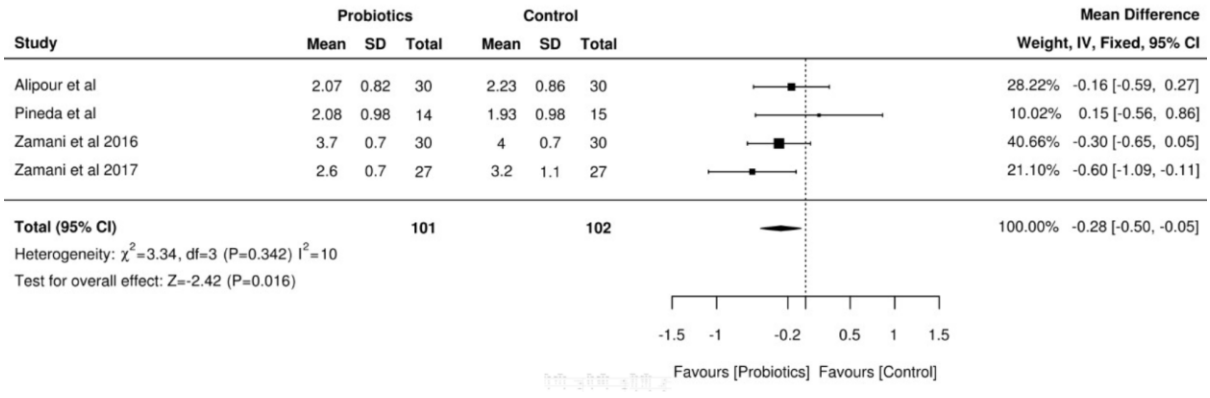
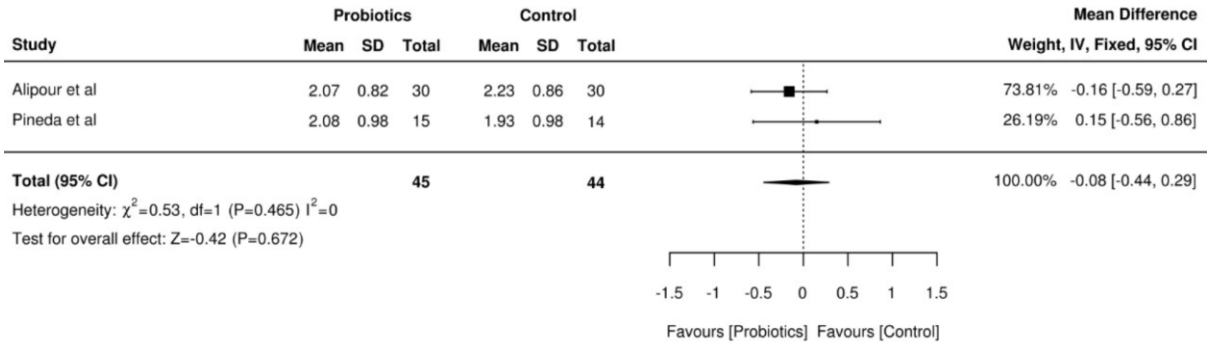


Figure 23 Forest plot representing the meta-analysis on 4 studies investigating the effects of probiotics upon Disease Activity Score (DAS28) score.

Sub population analysis revealed that studies using a combined formulation was statistically significant at reducing DAS28, effect size -0.4 (p=0.006) compared to Lactobacillus alone, effect size -0.08 (p=0.666), see Figure 24 a,b.

A) Lactobacillus



B) Lactobacillus and Bifidobacterium

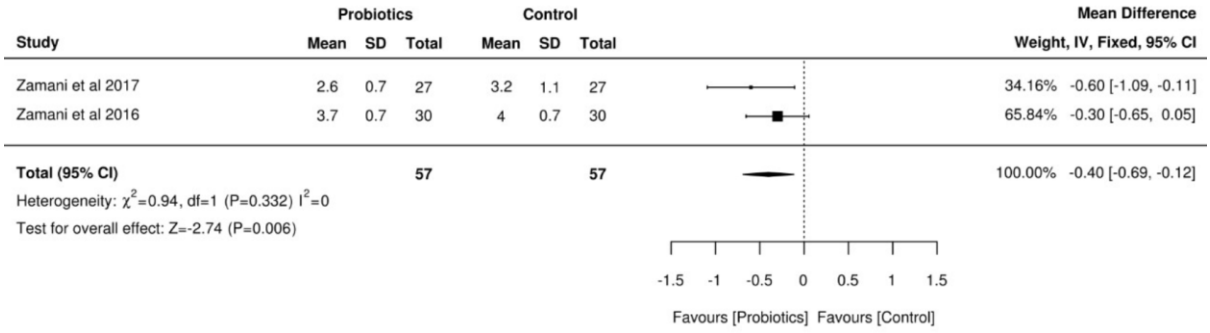


Figure 24a and b Forest plot representing the sub-group analysis to investigate the effect of probiotic formulation upon Disease Activity Score (DAS28).

A single study into patients with SpA by Tomasello et al.[226] assessed the composite score after employing two separate applications of four separate species in a high strength formulation. Analysing the average score obtained over the entire period of observation the score was significantly lower for the group receiving probiotics ($p<0.05$). However, during the first week of treatment, patients were given a mixture of *Enterococcus faecium* and *Saccharomyces boulardii* and in the second week they were given a mixture of *Lactobacillus salivarius and Lactobacillus acidophilus*. This unique intervention makes it harder to interpret which component of the formulation may be responsible for the overall reduction in score.

Disease specific composite outcome measures

Jenks et al.[221] employed aspects of the Bath Ankylosing Spondylitis disease activity index measures for individuals with longstanding ankylosing spondylitis after the intervention of a low concentration multispecies formula of three bacteria, (*Bifidobacterium lactis*, *Streptococcus salivarius and Lactobacillus acidophilus*). Whilst the calculated effect size in for both outcomes favoured probiotics (-0.1 and -0.6 respectively) both the confidence intervals crossed the midline therefore the effect is not significant. A similar result was provided when outcomes were measured using the ankylosing spondylitis quality of life measure, with an effects size reported as -0.5 (95% CI -2, 1.1).

Shukla et al.[225] employed a juvenile arthritis specific scale, in two formats using both the erythrocyte sedimentation rate and C-reactive protein. When comparing median changes after 12 weeks using the Mann Whitney U test , neither changes in score were statistically significant (p=0.06 and p=0.16 respectively).

Chapter Four Discussion

4.1 Introduction to the discussion

The use of probiotics for general health and wellbeing and the scientific appraisal of their effects has occurred for more than one hundred years. Recent advances in genomics and immunology has allowed a more nuanced appraisal of the relative benefits and risks of probiotics. Understanding their influence on the gut microbial communities, immune function and human health has indicated a possible role for probiotics in controlling inflammation in a range of auto-immune inflammatory conditions.

Prior systematic reviews have examined probiotics for rheumatoid arthritis. Pan et al.[202] and Rudbane et al.[203], both focused on changes in systemic inflammation outcomes (disease activity scores, C-reactive protein and cytokine expression), demonstrated an overlap in primary sources and both concluded that the statistically significant changes in systemic inflammatory outcomes may not reach clinical significance.
Mohammed et al.[204] revised the inclusion criteria and found nine studies for patients with RA, which when analysed, provided similar systemic inflammation outcomes and conclusions. The changes in cytokine biomarkers noted by Mohammed et al.[204] could not provide clinical conclusions. A further review by Dejoras et al.[205] was lacking detail as it was only published only in abstract form, however it used the same databases, population group, inclusion criteria and reached similar conclusions proposing that larger study sizes were required.

This review aimed to broaden the scope to include a wider range of inflammatory auto immune arthritis conditions. It also aimed to investigate in greater detail the formulations used in the clinical trials, in order to provide clearer pragmatic guidance to clinicians when discussing probiotics with patients. Whilst the medical community may be undecided on the validity of probiotic claims, patients are taking them in record numbers. Therefore, a sound understanding of the current state of evidence for their actions remains appropriate for rheumatologists. The systematic review was successful in finding 12 studies which could contribute to the knowledge base.

4.1.1 Structure of the discussion

The discussion starts with the summary of findings, see Table 25, followed by discussion relevant to each OMERACT outcome domain: life impact, disease manifestations and adverse events. This includes discussion of effect size, level of clinical and statistical significance. Sub-population analysis is reviewed, and space devoted to study demographics as recognizing sub-populations is the key to enabling risk/benefit stratification. Where appropriate, the effect of specific probiotic formulations are discussed including dosage, species number, species mix and prebiotic inclusions. The discussion concludes by reviewing the practical significance findings for future research and clinical practice.

4.2 Summary of findings

Table 25 Summary of Findings

Outcome		No participants, studies and duration	Certainty of the evidence (grade)	Effect size Comments	
Quality of life	PtGa HAQ	371 (6 studies) 56 to 365 days	⊕⊕⊕◯ MODERATE d,e	-0.37 SMD (CI -0.59, -1.5) P=0.001	Subgroup analysis revealed that sole Lactobacillus formulations more effective than combined.
Fatigue	VAS MAFS	93 (2 studies) 60-84 days	⊕◯◯◯ V.LOW a,b,c,e	-0.51 SMD (CI -0.93,0.09) P=0.018	Did not reach MCID. significant heterogeneity limits further conclusions
Bowel symptoms	DISQ VAS	596 (11 studies) 56-365 days	⊕⊕◯◯ LOW a,b,c,e	-0.04 SMD (CI-0.23,0.31) P=0.763	Did not reach MCID. Significant heterogeneity limits the ability to draw any further conclusions.
Pain	VAS	210 (3 studies)	⊕⊕⊕◯ MODERATE e.	-8.97 MD (CI-15.38,-2.56) P=0.006	Did not reach MCID.
Composite scores	DAS 28	210 (4 studies) 56-60 days	⊕⊕⊕◯ MODERATE d,e	-0.28 MD (CI -0.5,-0.05) P=0.016	Did not reach MCID. Sub population analysis revealed that a combined formulation was statistically significant at reducing DAS28 than Lactobacillus alone.
Systemic markers	CRP	325 (7 studies) 56-365 days	⊕⊕◯◯ LOW c,d,e	-2.34 MD (CI-4.26,-0.41) P=0.017	Did not reach MCID. Sub population analysis revealed a statistically significant decrease in CRP only seen in RA, and that a combined formulation was more effective at reducing CRP
Adverse effects	event	210 (2 studies) 84 days	⊕◯◯◯ VERY LOW b,c,d,e	1.02 RR (0.69,1.51) P=0.927	Many studies did not formally assess. Subpopulation analysis did not find any statistical difference between outcomes in RA and SpA.

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). MD: mean difference; SMD: standardised mean difference, MCID: minimally clinically significant difference, RCT: Randomised controlled trial GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect
Explanations a. small sample size ,b. varying estimates across studies, c. I^2>50%,d. sig risk of bias, e. Indirect comparison
PtGa Patient General assessment, HAQ (Health assessment questionnaire) Visual Analogue Scale, Multi-dimensional assessment of fatigue (MAFS) Disease activity score (DAS28) C reactive protein (CRP) Dudley inflammatory bowel symptoms Questionnaire (DISQ). Minimal clinically important difference (MCID)

4.3 Outcomes

4.3.1 Life impact

Quality of life

Effect scores calculated by the authors indicated a trend towards the improvement of patient reported quality of life, but this did not reach statistical significance in any individual study. Meta-analysis indicated a small but statistically significant benefit of probiotic administration. Statistically significant changes in quality of life, were not expected as most individuals in the included studies had a long disease duration prior to inclusion in the clinical trials. Therefore, they were likely to experience the consequences of longstanding disease, such as bony erosions, which may be contributing factors to disability but be considered relatively unamenable to change.

There have been other systematic reviews completed regarding the effects of probiotics on quality of life. A review on individuals with RA by Mohammed et al.[204] assessed the mean difference between two studies that employed the Health Assessment Questionnaire as an outcome. Due to high heterogeneity a random effects model was applied, and a statistically significant effect was not found, p= 0.081. The difference in findings between the Mohammed et a.l[204] and this review, may be related to the number of studies involved but also the greater proportion of unidimensional outcomes used in this review. Whereas Mohammed et al.[204] used a more complex domain questionnaire that includes a broader disability index. Whilst the disability index used by Mohammed et al.[204] aims to measure discrete physical limitation of daily activity– it is difficult to disengage physical limitations from the emotional impact of health conditions (such as helplessness), the impact of comorbid conditions and restriction in participation due to external environmental aspects. For all these reasons poor associations have been reported between calculated change in

disability and patient perceptions of disability and may underlie why a statistically significant difference was not found in the review by Mohammed et al.[204]

The author of this review suggests that probiotics may be moderating perceived quality of life indirectly through perception and mood, as some recent studies have shown decreased central responses to stress and negative stimuli after the application of probiotics and resulting changes in anxiety and low mood.[237-239] This research may provide a further step in clarifying how probiotics not only change brain activity but also human behaviours such as engaging in activities of daily living and perception of quality of life.

It is important that small statistically significant benefits are evaluated to determine the real clinical significance. Whilst a MCID has not been clearly stated for the many differently worded versions of the patient general assessment , the minimal clinical difference in rheumatology measures as identified by the OMERACT Rasch group, is 11 points on a 100 point scale .[240] Alternatively using two points as a minimal clinical difference would correlate to the recommended American College of Rheumatology response of 20% which identifies individuals as a responder to intervention. The second most commonly used outcome the Health assessment questionnaire has a stated minimal clinical difference of 0.22.[164] Examining the forest plot indicates that only Jenks et al.[221] and Hattaka et al.[220] provided a confidence interval that includes the minimal clinical difference for the scale used. Therefore, whilst probiotics can create a statistically significant change in quality of life measures, this remains only a possibly clinically significant finding.

The sub-group analysis of quality of life revealed a greater effect size from the use of Lactobacillus compared to combination Lactobacillus and Bifidobacterium. This may be an artefact of small study sizes as a recent review has found that many different formulations of probiotics (including combined Lactobacillus and Bifidobacterium) can be associated with a significant reduction in depression.[241] Underlying mechanisms have been hypothesised as the probiotics capacity to generate serotonergic precursor or serotonin type chemicals.[242] However the Lactobacillus only sub group displayed that the formulation of *Lactobacillus rhamnosus* and *Lactobacillus reuteri* appears more effective than *Lactobacillus rhamnosus* alone , and any combination of *Lactobacillus rhamnosus* is more effective than Lactobacillus caseii alone. A hypothesis to explain the lower effect suggested for *Lactobacillus caseii* may

relate to its absence of surface layer adhesion proteins, which reduce its capacity to attach in the gut and therefore its immunomodulatory capacity.[243]

Fatigue

Fatigue in rheumatology patients has been described as a "multidimensional, persistent symptom with far-reaching consequences".[140 (p.1)] Despite international consensus that fatigue should be evaluated in clinical trials for inflammatory arthritis only two studies included in this systematic review employed fatigue as an outcome. In both studies the post-intervention fatigue scores reduced. Calculating effects size from post test scores a small statistically benefit for the administration of probiotics was seen, effect size -0.51 p=0.018. However significant heterogeneity is shown by an I^2 of 83 indicating that the true size of effect is unknown, and that more data is required to clarify the impact of probiotics on fatigue. GRADE analysis also revealed a low confidence in the effects identified due to high risk of bias in studies. Due to low numbers of studies and heterogeneity any subgroup analysis to investigate differences between formulations was not appropriate.

The cause of rheumatological related fatigue remains debated and complex. For example, even in those patients who respond well and achieve remission with disease modifying anti rheumatic medications, only approximately a third achieve resolution of their fatigue symptoms.[136] A systematic review into the causality of fatigue by Nikolaus et al.[143] indicates strong relationships between fatigue and both pain and depression. Such links may provide a hypothesis for the amelioration of fatigue via the psychobiotic effect of probiotics on the gut-brain axis. Specifically, that probiotics can create a benefit in positive mental health.[244] The research underpinning this hypothesis has been largely based on animal studies, after the finding that germ free mice were found to have an exaggerated central nervous system response to stress, which could be altered by intervention with specific Bifidobacterium species.[245] Many subsequent studies have clarified that microbiota changes affect anxiety related behaviour in mice, for a deeper review see Foster et al.[246] Limitations apply when generalising results from murine studies, so further research is warranted to investigate the effect of probiotics on fatigue.

Bowel symptoms

Two studies investigated the effect of probiotics supplementation on bowel symptoms, both were addressing SpA. When calculating effects size from post-test scores without adjustment both study lines cross the line of null effect, therefore the plot does not illustrate a statistically significant result and significant heterogeneity limits the ability to draw any further conclusions. Both studies were undertaken on populations with long-standing Spondyloarthropathy. Gut inflammation in the SpA has been categorised into two main forms, an acute gut inflammation and a chronic form.[247] The chronic form has been associated with significant tissue changes, similar to that seen in Crohn's disease, that alter the normal function of the gut. Changes include detachment of epithelial layers, villi vacuoles and haemorrhagic extravasation.[247] Systematic reviews into the effect of probiotics in inflammatory bowel disease have shown a differential response between ulcerative colitis and Crohn's whereby probiotics have not been assessed as effective in adult Crohn's.[248,249] Therefore, it may be extrapolated that Crohn's like changes in long standing SpA would also be unamenable to impact by probiotics. Whilst subclinical gut inflammation has been described in up to 60% of patients with SpA the level and extent of damage to the gut in the populations included within this review remain an unknown confounding factor.[250] Future studies would require earlier intervention and more nuanced methods of assessing baseline gut inflammation.

Pain

Evidence has been provided, that gut microbiota can modulate key pathways that affect the pain experience. Meta-analysis indicated a positive and statistically significant effect of probiotic administration on patient reported pain using a visual analogue scale in RA. However, a meta-analysis using tender joint score indicated no significant benefit of probiotics. This latter result agrees with the recent review of Mohammed et al.[204] who looked at the data from five studies on RA who had included tender joint scores.

Identifying exact levels of clinically significant changes in visual analogue scale pain has been much debated. The smallest detectable difference on a measurement scale is the smallest change that can be reliably distinguished from random error. The smallest detectable difference reported in three randomised controlled trials of rheumatoid arthritis, given as negative change or improvement, was from−18.6, to −20.0. [136] Therefore the effect size in

this meta-analysis (-8.97) did not reach a level at which could be distinguishable from random error.

Minimal clinically important difference may also be used and should be appreciated as context-specific, for example a small change in very severe pain may be more significant to a patient than a larger change in low levels of discomfort.[235] As minimal clinically important difference is context specific, cautious interpretation and judicious application are required. Applying the latter part of Jaeschke's definition of minimal clinically important difference, it is important to consider what level of change in pain would "mandate a change in patients management".[124 (p.260)] At less than ten mm change on a 100 mm scale, it would be unlikely this would mandate management change and confirms it is not a clinically significant result. Furthermore if, as Graham et al.[251] suggests, a minimal clinically important difference cannot easily be converted between 10 and 100 point visual analogue scales, then applying it to this meta-analysis may be misguided. The effect size of all the studies included in the meta-analysis did not exceed the minimal clinically important difference of 10mm, therefore it would not be termed a clinically significant result. Assessing the impact of pain on individuals is not simple, an alternative approach has been suggested to use the percentage change from baseline. Using this approach, it is seen that wide variation in change as a percentage from baseline were reported. In summary whilst the interpretation of the significance of the result upon pain is complex and depends upon the methodology applied, the small statistically significant difference did not reach clinical significance in any of the methodologies applied.

4.3.2 Adverse events

There are many characteristics required of effective probiotics, for example the capacity to survive passage of the upper gastrointestinal tract, to persist and to interact with the mucosal layers that create immunological responses. However, most importantly they should be safe, which includes being non-pathogenic, not capable of transferring antibiotic resistance and demonstrated not to cause any other harms. Serious side effects were uncommon in this review. A prior systematic review into the safety of probiotics by Didari et al.[44] focused on major side effects and identified bacterial sepsis, as the main issue in high-risk groups such as the immune-compromised patient. Despite many patients in this review

taking systemic steroids and/or disease modifying anti-rheumatic diseases and being considered clinically immune compromised higher risk of adverse events was not demonstrated.

The risk of a minor adverse effect from each study, calculated taking an intention to treat approach, varied from 1 to 1.28 which indicates approximately the same number of events being reported by individuals in the control group as in the intervention group.

Table 26 Side effects as categorised according to WHO[85]

Classification	Symptom severity	Modification of treatment	total probiotics	Total control
Grade 1	Asymptomatic or mild	Observation only	16% 33/211	15% 31/206
Grade 2	Moderate	Minimal non-invasive intervention or limitations of ADL	0.5% 1/211	0.5% 1/206
Grade 3	Severe	Requires hospitalisation and disabling	0	0
Grade 4	Life threatening	Urgent intervention required	0	0
Grade 5	death		0	0

There was a trend that more studies reporting minor adverse effects concerned individual with SpA. Sub-population analysis did not reveal a statistically significant difference, but this trend is a new finding. It may represent an increased sensitivity of the gastrointestinal tract to administered probiotics in SpA conditions or better assessment and reporting of bowel related side effects in spondyloarthritis as opposed to rheumatoid arthritis. Consequently, number needed to treat (harm) concerning minor side effects was higher in the trials concerning SpA patients.

There are many problems when attempting to include adverse events in meta-analysis, when the original trials concerned have not used these as a primary endpoint. Issues include incomplete reporting, inconsistent definitions and an unknown level of vigilance regarding adverse events.[252] The number of trials with brief information or lacking a discussion of adverse events in this review provide a high index of suspicion of low vigilance.
The wide variation in trial time spans for the studies in this review (from 7-365 days) may also be problematic if, as stated, that “the hazard of an adverse event is not constant over time… then percentages reported from trials with varying follow-up periods may generate a misleading estimate of the adverse event”.[252 (p.107)] Whilst the failure to include zero events

in relative risk meta-analysis is common, the approach taken to avoid this pitfall in this thesis was to assign a very low numerical value to zero event trials equally to intervention and control arms. This allowed the software SUMARI (System for the Unified Management, Assessment and Review of Information) to calculate a forest plot for relative risk. It should be noted that there were significant population exclusions employed in all the included trials that may underestimate the true risk of probiotics being applied in the wider rheumatological population, for example almost all studies excluded individuals with renal or liver disease. An intention-to-treat concept including the sample numbers originally recruited was used in this review , as whilst it does not reflect actual probiotic exposure, making it harder to attribute adverse events to relevant underlying biological mechanisms an 'as treated' concept would have reduced randomization and small sample sizes even further.[253]

Whilst serious adverse effects related to the ingestion of the probiotics intervention were not widely reported it should be noted that both genera have known antibiotic resistance, Lactobacillus, most notably, to vancomycin and Bifidobacterium, most notably, to mupirocin and both have the capacity to share this resistance in the gut.[254] Commercial product screening for antibiotic resistance has occurred in some jurisdictions .[255] Such safety screening is advised if larger scale trials be considered. The results of this review agree with recent research that "Harms reporting in published reports of randomised controlled trials assessing probiotics, prebiotics, and synbiotics is often lacking or inadequate" and hopes that by raising the profile of this issue in this review, future work can be improved.[256 (p.240)] Taking all these factors into account the results from this study may be interpreted as highlighting a potential subgroup (spondyloarthropathy) with a different adverse effects profile to RA, and that further exploration is warranted.

4.3.3 Markers of systemic inflammation

C-Reactive Protein

Seven studies were included in the meta-analysis of effects of probiotics on C-reactive protein. Individual study results indicated a trend for probiotics to reduce C-reactive protein and, when combined in a meta-analysis, a statistically significant effect size is observed with low heterogeneity. This agrees with two recent systematic reviews and meta-analysis on the

effect of probiotics on C-reactive protein for rheumatoid arthritis. Pan et al.[202] included four studies on rheumatoid arthritis populations, identifying a statistically significant reduction in the levels of C-reactive protein (p= 0.007) and Mohammed et al.[204] also identified a statistically significant reduction (p=0.001).

Investigating SpA sub-populations did not reveal a benefit of probiotics on C-reactive protein levels, and there was high heterogeneity as shown by I^2=83 indicating that meta-analysis is not appropriate for these studies. Whilst interpretation of these results from limited studies should be cautious it does suggest a differential response between RA and SpA to common probiotic formulations may exist.

Sub-population analysis targeting different formulations of probiotics revealed a statistically significant benefit was only obtained by the mixture of probiotics (Lactobacillus and Bifidobacterium) compared to Lactobacillus alone. There is some research which may contribute a hypothesis for this finding. *Lactobacillus rhamnosus GG* has been shown to raise key elements in the inflammatory pathways, specifically interleukin 10, also known as human cytokine synthesis inhibitory factor, when assessed in murine studies.[257] This key regulatory cytokine is a complex player in innate and adaptive immunes responses. Interleukin 10 may be produced by many different types of cells and they can interact in feedforward and feedback pathways determining not only the volume of circulating cytokine but also what function it performs. Research has suggested that all three elements, the feedback loops, the source and timing of secretion of interleukin 10 is vital in determining an anti-inflammatory outcome and long-term immune regulation.[257,258] It is this complex and contradictory action of interleukin 10 that has made it hard to harness as a pharmaceutical intervention and the result of this study could suggest the interplay of other microbiota may also be a determining factor in the capacity of interleukin 10 to down regulate (not up regulate) the inflammatory pathways.

Caution interpreting the results of this systematic review on C-reactive protein are advised as the significance of changes may be conditional on the baseline from which the change occurs. When patients with baseline low levels of C-reactive protein are included ,such as in this review, it is difficult to estimate whether a small change in score remains more or less significant to the individual. It is also hard to evaluate the benefit of probiotics for patients

with low levels of systemic inflammatory markers, as this may not reflect the stages at which patients may consider using probiotics. There were no eligible studies which included patients with C-reactive protein in the moderate or high range at baseline. In fact, two of the included studies had baseline values within normal range, this was seen in the probiotic group in Hattaka et al.[220] and control group of Shukla et al.[225] Considering this low baseline, the author of this review suggests that a potential role exists for probiotics in maintaining remission via C-reactive protein maintenance, or reduction, as no evidence exists for probiotics roles in acute and high inflammatory C-reactive protein states. It is known that "remission rates, particularly off-therapy, remain low, and a long-term cure, or re-establishment of immune homeostasis, is elusive for all but a minority."[259(p.63)] Therefore enabling patients who are in 'off medication' remission to access appropriate probiotic regimes could be an interesting and ethical approach to gauge the contribution of probiotics to long term suppression of inflammation and overall disease management. Significant variance exists in the definition of remission and estimates of successful remission between different conditions and across different age groups. As there are no current studies which have specifically investigated any role of probiotics in remission it remains a fertile area for further investigation.

Cytokine profiles were not included in this review, for they are not yet core OMERACT outcome measures and a complete understanding of specific cytokines in inflammatory arthritis has not been achieved. There is appreciation that the method of profiling can exert considerable effect on outcomes, in research settings, and that their role as reliable biomarkers remains unclear due to the confounding effects of age and gender.[104] Defining the exact nature of condition specific cytokine changes or pathological microbial signatures remains a work in progress.[260]

Composite Disease activity Score DAS28

Meta-analysis revealed a statistically significant effect of probiotics on disease activity score. However, as clinically significant reductions in this score when using C-reactive protein, are estimated at a drop of 1 point overall, the combined effects size did not reach clinical significance. This agrees with prior reviews as discussed in the introduction.[206,208] Disease activity scores are calculated using a formula which weights the different individual elements. It is worth examining how each weighted element can affect the final score in

order to identify any factors that may have affected disease activity scores calculation for the studies within this review. Firstly, variation in wording within the patient general assessment component can create meaningful differences of up to 0.63 in final score, however as study populations included in this review had small changes in patient general assessment scores minimal impact of wording variations is expected.[174] Secondly measures of systemic inflammation (such as C-reactive protein) are expressed as a log function, therefore small changes can provide a large change on the final composite score. As the studies included within this review generally displayed small changes from a low base of both C-reactive protein and tender joint scores, this could cause a proportionally higher impact on final score. It should be noted that some studies could not be incorporated into the meta-analysis because of a lack of raw data, but as these studies were included in the reviews by Pan et al. and Mohammed et al., it is unlikely to change the agreement between all three reviews.[202,204]

Sub-population analysis of the available studies, with appropriate data, revealed that a combined formulation of Lactobacillus and Bifidobacterium created greater effect sizes than Lactobacillus alone. This is discussed further in section 4.5.1.

4.4 Heterogeneity of included studies

The inclusion and exclusion criteria for studies were pre-specified, as per the systematic review protocol. However, significant levels of heterogeneity remained in population demographics (for example age, disease duration, and applied exclusion criteria) and intervention (for example probiotic formulation, strength and dosage). These elements and their potential influence on the outcomes are discussed below.

4.4.1 Confounding factors

Condition

Results indicate some condition specific responses, specifically the greater likelihood of adverse events and reduced anti-inflammatory capacity of probiotics as measured via reduction in C-reactive protein. As subclinical intestinal inflammation occurs in a significant number of patients living with SpA and “it has been demonstrated that up to 10% of those patients displaying chronic gut inflammation will develop over the time a clinically overt CD” this may explains the differential findings between RA and SpA patients.[247(p.2,)] The

timelines for a transition from acute to chronic changes in the bowel have not been determined, but it would be logical to expect that the individual with longstanding disease duration may be more likely to experience such changes and therefore may also have altered interaction with probiotic formulations. Using biopsy specimens along the gut may create a more nuanced picture of dysbiosis for individuals with SpA however, as this invasive action is unjustified, this review would indicate that both patients and health professionals should be aware that individual with SpA taking probiotics may experience more minor side effects and experience smaller reductions in markers of systemic inflammation.

Age

There is significant interpersonal variation in the microbiome, however healthy young adults have been demonstrated to have a relatively stable personal composition of gut microbiota.[261-263] in comparison older adults (over 65) have more interpersonal microbiota variability, lower overall levels of diversity and recognised to experience 'immunoscenescence'. This is when the immune system is compromised leading to a chronic low grade inflammatory state.[264] Studies have shown that elderly patients often specifically lack Bifidobacterium species and that supplementation with these can increase the size and diversity of Bifidobacterium populations.[265] Therefore studies that include a greater proportion of older patients may show a different response to formulations with Bifidobacterium. Interestingly the two studies with the oldest age populations, Mandel et al.[223] average age 62.9 yrs. and Pineda et al.[224] , average age of 63.8 years only supplied Lactobacillus formulations.

Only one study involved patients under the aged of 16.[225] Studies have indicated that the juvenile arthritis microbiome similarly shows a relative lack of the family of firmicutes bacteria (including, but not limited to Bifidobacterium).[266] The study included in this review provided a variety of Firmicutes species in a mixture known as VSL#3 . VSL#3 is a high-concentrated probiotic of eight different bacterium that has demonstrated efficacy in the control of inflammation in other conditions, for example extending the remission in inflammatory bowel disease[249]. Future results will be interesting and large international clinical trial is currently recruiting juvenile arthritis patients to investigate the impact of this probiotic product.[267]

Disease duration

Inflammatory arthritis has a long prodromal period, and the earlier that diagnosis and intervention can occur, the better the likelihood of disease remission and symptom reduction.[268] This has led to the concept of a 'window' of opportunity for intervention, in the first three years post diagnosis. All study populations had a mean duration of greater than three years, indicating established disease status beyond the window of intervention. Three studies included participants with a mean disease duration of greater than ten years. Therefore longstanding changes in multiple systems, including but not limited to structural joint damage and changes in central processing of pain may be expected. Research in RA has discovered that both the type of cytokines involved in generating auto immune dysfunction in early RA and their mechanism of action seem to be different from those that are involved in persistence of the disease long term.[259] Synovitis established for more than six months is harder ro resolve and supports the theory of a switch from cytokines initiated from gut immunity challenges to cytokines created from fibroblasts in and around the joints. Consequently, it may be hypothesised that probiotics have a different capacity to influence established auto immune disease.

Relatively little is known about when Australian patients access complementary treatment in their arthritis journey. A study into the complementary therapy use of American patients with RA discovered that therapies were frequently used in the early stages of disease alongside conventional treatments.[269] However this is contrary to the disease period in which clinical trials of probiotics have been conducted. The average disease duration was significantly longer in the studies of patients with SpA than compared to RA. This is significant as early use of anti-TNF treatment in peripheral spondyloarthritis (pSpA) has demonstrated more than 50% of patients can achieve sustained drug-free clinical remission and suggests that later disease is marked by loss of tolerance, epigenetic modifications, or irreparably impaired immunoregulatory pathways.[270]

In summary, the current research is conducted mainly on patients with extended disease duration, missing the window of opportunity for immunological change via the gut and does not provide transferable results for consumers taking probiotics early on their disease journey.

Body weight

Four studies excluded individuals on any type of formal 'diet' but only two excluded individuals with a body mass index that placed them in the morbidly obese category (score >40). Therefore, by default, overweight or obese individuals were included within all studies. Conflicting results surround the influence of obesity on the microbial community, but it has been characterised as showing reduced diversity and altered Firmicutes:Bacteroides ratio.[271,272] Zmora et al.[273] have reviewed the role of obesity in the pathogenesis of RA and identified that body mass index becomes significantly associated with inflammatory markers and is relevant to the inflammatory state as the disease progresses. Therefore including patients with a long disease duration and obese status adds another confounding factor to the included studies.

Whilst meta-analysis has shown probiotic administration can provide a small effect size for weight loss in otherwise healthy individuals, the question of how an individuals body mass index may affect probiotic intake has not been so thoroughly examined[274]. Relationships between obesity and inflammation, are yet to be fully clarified but the evidence of a persistent, low-grade, inflammatory response in overweight and obese individuals may suggest sub population specific responses to probiotic intervention dependant on their baseline weight. Whilst this review did not find any relevant probiotic studies applied to patients with psoriatic arthritis there is a known relationship whereby obese people with psoritaic arthritis are 48% less likely than their normal-weight counterparts to reach, after a year, a point of 'minimal disease activity', and the heavier the individual the less likely they were to respond to pharmaceutical management.[275] Therefore the affects of probiotics for patients of different body weights with inflammatory arthritis cannot be presumed. Recent surveys from the United Kingdom have identified that the most significant change in individuals presenting with RA is the comorbid burden of obesity, with obesity prevalence in newly diagnosed individuals rising from 13.3% in 1990 to 33.6% in 2010.[276] Therefore considering the affect of obesiogenic inflammation will become an increasing priority for clinicians and future probiotic trials should better account for body mass index.

Diet

Research indicates that dietary influence can be an even greater influence on the gut microbiome than body mass index, in RA.[277] Higher levels of Bacteroidetes phylum are

associated with a highly processed food diet, higher levels of firmicutes are associated with a greater fresh food intake and high longterm carbohydrate intake is associated with prevotella bacteria.[273,277] The impact of different culture and diet therefore may be a confounding factor when assessing probiotic intervention. The studies within this review came from different socio economic and cultural regions see Figure 26, therefore heterogenous dietary patterns are likely

Study	Country
Alipour et al[218]	Iran
Brophy et al	UK
Hatakka et al[220]	Finland
Jenks et al[221]	New Zealand
Lee et al[222]	New Zealand
Mandel et al[223]	New Zealand
Pineda Mde L et al[224]	Canada
Shukla et al [225]	India
Tomasello et al [226]	Italy
Zamani et al[228]	Iran
Zamani et al[229]	Iran

Figure 25 Geographic location of studies incorporated within the review, with marked numbers of studies per location.

In conclusion tighter demographic control or capacity for sub-population analysis is required to pull out the confounding factors of diet and body weight. As simple food-frequency questionnaires, strongly associate with core microbiome status, future studies may reduce the confounding factors of diet by incorporating their use.[278]

Concurrent Medications

Probiotics were supplied as adjunct medications in all the included studies. Gut commensal bacteria actively participate in the metabolism of many chemical compounds, thereby potentially impacting drug availability, levels, and toxicity. Conversely medications, may impact upon the presence, function and viability of gut microbiota. The most commonly used medications, within the studies of this review, are discussed below.

Non-steroidal anti-inflammatories were stated as available for participants in the majority of studies, and formally excluded in two studies.[227,236] Non-steroidal anti-inflammatories can reduce symptoms of joint pain and swelling and their use may confound overall outcomes. Only Brophy et al.[219] identified statistically different percentages of this medication use at baseline between groups ,with 85% of probiotic intervention group and in 65% of the control group using non-steroidal anti-inflammatories. Murine research has identified that probiotics can have a complex relationship with non-steroidal anti-inflammatories.[279] Dependant on the diversity and ratios of bacteria present, the microbiota can ameliorate the adverse enteropathic affects associated with this medications use, but can also alter drug metabolism such that medication efficacy is also changed.[280,281] Where Non-steroidal anti-inflammatories usage was employed as a formal outcome measure, no reduction in the usage or difference between control and intervention was observed.[225] The lack of personalised microbial analysis within the sample populations of the included studies precludes the ability to understand how all these complex links may have changed study outcomes. However, the possible confounding influences should be acknowledged in studies where recent antibiotic use and current non-steroidal anti-inflammatories use is occuring.

Antibiotics. It is generally accepted that the short term effect of antibiotics is a significant loss of microbial diversity and 16S rRNA gene sequencing techniques have shown that broad-spectrum antibiotics also change the bacteroidetes:firmicutes ratio.[282] Greater detail is provided in a recent review by Mikkelsen et al.[283] Therefore, it is relevant that five of the studies included in this review did not state recent use of antibiotics as an exclusion criteria and this may be a confounding factor.

Steroids. Participants taking oral corticosteroids were excluded in the study by Shukla et al.[225] not stated in four and as allowed in the other studies. Corticosteroid medications are anti-inflammatory immune suppressants and as such can reduce the symptoms associated with Inflammatory arthritis. They are quick acting and could be a confounding factor in the change in symptoms noted over the course of the trial however it was stipulated that medication use should be stable and there was no statistical significant difference in precentages taken between control and intervention groups.

Intra-articular steroids can provide rapid relief of discrete joint pain and swelling. These were only stated as allowed in two studies, Jenks et al.[221] and Hatakka et al.[220] Double the number of participants in the probiotic group received steroid injections in Jenks et al.[221] compared to the control group raising the likelihood of this being a confounding factor.

Disease modifying anti rheumatic drugs (DMARDS) Traditional diseae modifying ant rheuamtic medications, which include methotrexate, leflunomide, hydroxychloroquine, and sulfasalazine reduce the immune responses that contribute to pain and inflammation in a broad manner. Several known mechanisms exist by which the intestinal microbiota can either directly, or indirectly, change the bioavailability and/or efficacy of such drugs. It is known that there is considerable individual variation in response to drug treatments, for example, methotrexate has highly variable response rates suggested to relate to gut microbiota.[282] As methotrexate, as similar medications,were allowed in eight of the included studies it may be considered a confounding factor. The study by Lee et al.[222] looked specifically at the effect of probiotics upon patients taking the anti-rheumatic medication Sulfasalazine, which requires specific intestinal microbiota to convert it in to its active form in animal studies.[284] Short-term treatment of RA patients with a multi-strain probiotic was not found to statistically influence Sulfasalazine in this study although the authors identified a large interpersonal variation in drug metabolism at baseline which would obscure any small effects from this short study.[222]

In conclusion heterogenity of concurrent medications and paucity of considerations of their affect on the micorbiome and ingested probiotics is a limiting factor in this review.There were no studies in which the newer generation biological or small molecule diseae modifying anot-rheumatic medications were taken alongside probiotics, therefore the findings of this review with regards to tolerability, safety and efficacy of probiotics cannot be generalised to individuals on these next generation medications.

Duration of intervention

The median duration of delivery of probiotic interventions was 84 days. Little is known about the required duration for probiotics in rheumatology or the persistence of any effects when their administration is ceased. Studies from other areas of investigation can provide some guidance. Murine studies have indicated that the protective effects of probiotics

against non-steroidal anti-inflammatories enteropathy require a minimum of a weeks duration of probiotic supply prior to medication use.[285] The optimal duration of supply is likely to depend upon the mechanism of effect. If proposing that probiotics are only working through competitive exclusion of more pathogenic or pro inflammatory bacteria, this presupposes that mucosal attachment, long term persistence and colonisation within the relevant part of the gastrointestinal tract is required to maintain the benefit. Considering that individual probiotics may have limited persistence in the gut then ongoing ingestion would be required to maintain benefits. Persistence studies have been carried out for specific strains of Lactobacillus and Bifidobacterium, and demonstrated diverse resistance to bile, antbiotics, and variable adhesive properties.[243] The combined properties of multi-species probiotics have not been as fully investigated but it may be that broader range formulations have a potential for more diverse range of persistence and adhesive qualities of the probiotics included.[286] It should be noted that faecal recovery studies have been traditionally used to assess probiotic survival and persistence.

Alternatively if immune modulation, through the innate and acquired immune system, are the primary mechamisms it remains possible that, similar to disease modifying anti rheumatic medications, long term benefit can be achieved with a correctly timed intervention and that benefits may be maintained without ongoing administration.

When considering the alternative hypothesis, it is unfortunate that the studies in this review are not well placed to inform knowledge on persistence, due to the variation in probiotic intervention timelines and because just one study, Lee et al.[222], provided re analysis of outcomes after a three week wash out period. The results of Lee et al.[222] indicated no change after intervention or wash out in faecal species which may be interpreted in various ways. It may indicate an intrinsically stable intestinal microbiota, or insufficient probiotic formulations or instability of the faecal analysis to identify probiotic effect. A recent study by Zmora et al.[287] informs this discussion, as it supplied eleven bacterial species and all dropped to insignificant faecal levels after cessation of supplementation yet submucosal samples showed nine of the eleven had enriched the mucosal layers. The study reported that 'permissive' host microbiome features (for example HLAB27) could be used to estimate individual host susceptibility to probiotics modification but that invasive mucosal sampling is

the most accurate method of determining probiotic persistence. Therefore this review recommends that future studies work to identify further characteristics of permissive individuals and employs subpopulation stratification based upon these host and microbial features.

Probiotic formulations

Concentration The definition of probiotics states that organisms should be supplied in an adequate number to provide health benefits. The exact concentration required, however remains under debate. It has been estimated to be somewhere between 10^7 and 10^9.[288] A clear dose dependant relationship has not been demonstrated for probiotics. Establishing suitable concentrations for multi strain formulations has been even less investigated than for single strain formulations, however a study concerning a four strain probiotic by Taverniti et al.[289] reported that the higher concentration 70 billion dose, compared to seven billion dose, did result in earlier, larger and longer durations of viable bacteria being found using faecal monitoring. This suggests that a healthier number of bacteria surviving the stomach and entering the intestines will provide higher benefits to the individual. However, using faecal bacterial load may be misrepresentative of the microbe's ability to deliver health benefits, as bacteria may be transient passengers in the gut lumen with greater faecal shed relating only to higher ingestion rates, not to replication, persistence or immunomodulation. Historically work has focused on determining probiotic concentrations required to survive stomach and intestinal passage, as opposed to concentrations required to ensure optimal mucosal contact and immunomodulation. Significant differences in the concentration of probiotic formula, as measured by colony forming units, were found across the included studies. Interestingly, murine studies into the optimal therapeutic dosage of *Lactobacillus acidophilus* for ileal colitis identified 10^6 as providing better outcomes than stronger 10^8 dosages.[290] Therefore it cannot be assumed that higher concentrations will provide higher benefits. Recent research by Zmora et al.[287] has identified a significant inverse correlation between initial levels of a given probiotics species in a specific area of the gastrointestinal tract and its fold change. That means that low abundant species were more likely to expand than those already present in high loads. This may indicate a loading ceiling of capacity in the mucosal fold beyond which further probiotic supplementation will not change the composition, and therefore reinforces the concept that higher

concentrations may not always yield greater benefits. A probiotics monograph for industry stakeholders in the food industry, developed by Health Canada, lists a variety of recommended dosages for different species from 1x 10^7 to 3 x 10^8 in food.[88] However, the stipulation of specific concentrations for multi-species probiotic has not been established for healthy individuals or individuals with inflammatory arthritis. Therefore, it is hard to interpret the significance of the varying concentrations used within this review or provide practical guidance for a recommended concentration, in colony forming units, to clinicians or patients.

Viability The presence of live specific probiotic species and viability of these organisms in a given probiotic product is affected by many possible elements within the manufacturing process and thereafter during storage by the consumer.[291] Only the studies by Shukla et al.[225] and Brophy et al.[219] provided consumer instructions for refridgeration and product care, however there was no verification of this or checks made on the ongoing viability of the products they were supplied. Changes in viability to probiotics, due to failure of appropriate storage at multiple stages of the supply chain, and lack of verification of viability is a considerable source of error.

Species Verification There was no stated verification of the probiotic products supplied in the papers, except where the name of the commercial product was supplied. The integrity of commercial probiotic products cannot be assumed. Verification of commercial products by Goldstein et al.[292] identified that most products were correctly labelled however a larger review revealed a significant disparity in both content and concentration with up to a third of products containing insufficient viable cells to obtain any health benefit.[293,294] Lewis et al.[295] investigated 16 commercial Bifidobacterium products and found frequent differences between label and content which included misclassification of species, inclusion of additional species, variation in species between product batches and even within capsules within batches.

Classification Bacterial classification remains complex and changeable as discussed previously in chapter 1. This shifting taxonomy of probiotics creates unintentional disparities, for example, as identified by Mill et al.[296] the strain of *Bifidobacterium infantis* in the product VSL#3 has been more recently been reclassified to *Bifidobcaterium lactis*.[296] A

further example, within Tomasello et al.[226] identified the probiotic as *Saccharomyces boulardii*. Modern Srna technology has recently reclassified *Saccharomyces boulardii* as a variant of *Saccharomyces cerevisiae*.[297] It is recognised that accurate and proper nomenclature and strain specification is desirable for safety, repeatability and reliability but as detail was lacking in many studies a pragmatic approach of of investigating the the two main genera of probiotics was applied. It is recommended that future studies should be better designed to provide accurate detail on strains and therefore their proven mechanisms of action.

Availability for consumers Two commercial products were used within some studies in the review, VSL#3 made by Actial Farmaceutica and a BLIS technologies oral tablet. Only the VSL#3 remains available as BLIS have moved in to the development and marketing of oral probiotic prducts (lozenges, mouthwash and toothpaste) specifically for the paediatric market. The literature on the oral BLIS products was not included in this review as it was targeted to the prevention of Streptococcus throat infections, otitis and halitosis and did not target individuals with any forms of inflammatory arthritis.[298,299] However individuals with inflammatory arthritis display higher levels of periodontal disease there is considerable scope for further investigation of oral probiotic products in rheumatology patients.[300,301]

4.5 Study limitations

4.5.1 Strain specific mechanisms of action

Systematic reviews of probiotics face unique issues, as studies often utitlise a combination of probiotic formulations, in the absence of a rigorous evidence base for the underlying mechanisms of action of any given single component.[210] This may create heterogenous interventions inappropiate for systematic review or meta-analysis. Experts in the field have expressed the opinion that combining results on multi probiotic blends can occur if any of the following criteria are established: evidence that outlines some similarity in the blends mechanisms of action, a common physiological effect previously proven by at least one human study of quality, recognition of common structural basis, common secreted products or the capacity to grouped into logical subsets, including by genera.[210 (p. 5)]

Prior systematic reviews in this areas have largely avoided the consideration of limitations related to the content of the probiotic formula. This thesis has taken a pragmatic approach

based on the evidence for shared mechanisms and differences that may be relevant to the two broad genera of Lactobacillus and Bifidobacterium as outlined by Sanders et al. [64] Whilst they are taxonomically distinct groups, with the genus Lactobacillus sitting in the phylum Firmicutes and the genus Bifidobacterium in the phylum Actinobacteria they are both non-spore-forming, gram-positive, bacteria. Both genera are mucin binding bacteria which provides a shared capacity for them to attach to the mucosal lining of the gut, provide competitive exclusion to other pathogenic bacteria and interact with the host immune system, as opposed to being transitory members of the microbial community in the gut lumen. However, again they differ in the manner of this attachment with Lactobacillus displaying specific surface layer adhesion proteins that tend to create specific immunomodulatory affects. Both genera can stimulate the synthesis of key cytokines in the inflammatory pathway however usually via different mechanisms as they differ in the end product of their metabolic process which is lactic acid for Lactobacilllus and short chain fatty acids (SCFA) via a process known as the bif shunt for Bifidobacterium.[302] The resulting SCFA end products, affect synthesis of specific inflammatory cytokines, including inhibiting the synthesis of pro-inflammatory cytokines as well as promoting the production of the regulatory cytokine interleukin 10, as summarised in the review by Vlasova et al 2016.[303] Comparative genomic analysis has identified that the Bif Shunt pathway is shared across all species of Bifidobacterium, providing a rationale for grouping Bifidobacterium in subpopulation analysis.

Both genera of probiotics have been found to generate beneficial substances for humans such as vitamin B12 and folate, essential substances for mental health and wellbeing.[81] Studies suggest that low folate and low vitamin B12 are found in depressed patients, and there is “an association between depression and low levels of these two vitamins in studies of the general population”.[304(p.59),305] Supplementation of B12 and folate has been suggested to combat this deficiency, therefore providing probiotics capable of generating these substances in vitro may provide similar benefits in elevating mood, reducing anxiety and alleviating depression. This provides a rationale for grouping genera together when reviewing the probiotic effects on quality of life and pain.

The author of this review believes that the conditions for undertaking a systeamtic review are met due to identified shared mechanisms identified. Sanders et al.[64] recommend that where substantial differences are seen in study effects, specifically when study effects are shown in opposing directions, then sub grouping logically by the nature of the probiotic supplied should be considered. The author of this review considers that the conditions for meta-analysis specifically regarding the creation of logical subsets have been met and were justifiied given that many outcomes displayed substantial differences in effect size and direction across included studies.

Whilst the limitations imposed by the hetereogenity and mixed mechanism of probiotic formulations may reduce confidence in findings from this review, the author believes that investigating probiotics with some leeway to account for mechanisms of action is currently justified given the volume and frequency of usage by patients and emerging knowledge base on mecahnisms of action. Whilst the combined interaction of multi strain and multi species probiotic products is not yet understood and may be critiqued for adding extra complexity into the known mechanisms of action, there is also an opinion that providing mixtures of probiotics maximises the different unique properties from each strain and provides a greater range of benefits to the individual.

4.5.2 Drop out

Drop out rates above average and evidence of differential attrition between experimental and control groups were identified in some studies. Whether differential or equal, identifying the rationale behind drop out remains vital when considering bias. For example Alipour et al.[218] counted patients as 'drop out 'when excluded for not precisely following the protocol, which would be an example of missing in a non-random fashion, that may potentially affect outcome and be a source of bias. Alternatively, Brophy et al.[219] included 'drop out' that occurred early, prior to starting the intervention, for example, due to individuals moving overseas. This can be described as missing completely at random, as systematic differences between such drop outs and the patients who remained in the study are unlikely.[306] When using this taxonomy of missingness five of the included studies included drop outs missing in a non-random fashion, two as a mixture and only one as purely missing completely at random. Therefore, despite the overall equality in attrition, the potential for bias remains.

4.5.3 Adherence and compliance

Adherence rates across the studies varied substantially. Judging adherence and compliance can be complex. Only the internet based study by Brophy et al.[219] replied purely on self report. Tablet count was used by the majority of studies, but this remains open to patient based manipulation. Two studies used faecal count as a surrogate marker for probiotic adherence. The study by Hattaka et al.[220] identified an increase of Lactobacillus group bacteria in the intervention group from 25% at baseline to 83% post intervention, whereas the control group dropped from 23% to zero. The study by Lee et al.[222] did not identify faecal *Bifidobacterium lactis* after intervention. Traditional interpretation of these results may indicate that *Bifidobacterium lactis* did not survive passage, or colonised the gut. This may lead to an assumption that the patient would only benefit from those bateria found in faecal analysis. Recent research has suggested interpretation errors if relying on faecal analysis.[287] By comparing faecal count to lumen bacterial levels and intestinal biopsy Zmora et al.[287] confirmed that gut mucosal microbiome only partially correlates with stool as humans exhibit very specific patterns of 'permission'or 'resistance' to probiotic colonisation.[287] The authors concluded that faecal presence cannot determine if faecal presence of bacteria represents active colonisation or a passive 'wash out' of luminal bacterial contents. This may explain why systematic reviews have disagreed on the alteration in fecal microbiome. If it is only species bound in the mucosal layers and interacting closely with the epithelial surface that can engage in active immunomodulation then only gut biopsies can provide acurate microbiotia information and identify patient adherence to intervention.[287]

In conclusion, significant heterogeneity of the studies involved in this review provide numerous limitations regarding the population with respect to gender, condition, age and disease duration and heterogenity regarding the intervention with respect to probiotic dosage, formulation and application. Study probiotics were administered across a wide variety of time frames and in conjunction with a variety of medications. Sample sizes across the included studies were generally small, and therefore determining the clinical meaning of outcome measures that have not been validated for use in these patient groups is difficult, for example the use of the visual analogue scale for irritable bowel syndrome which has not

been validated in rheumatology patients. Lastly, there remains resonable doubt about the probiotic formulations supplied due to the lack of product batch verification.

4.6 Limitations of the review process

Only studies in English were included in this review, which introduces a potential source of bias and the risk of invalid conclusions should important studies be excluded. However as seen from Figure 26 included studies were undertaken across a range of English and non English speaking countries. Whilst all quality appraisal was undertaken by two reviewers and utilised a third when necessary to resolve differences, the initial study screening for inclusion and exclusion was undertaken by a sole reviewer, and the final data extraction was undertaken by a sole reviewer introducing the risk of human based error. The most significant limiting factor for this study was the variation in data and the presence of missing/ incomplete data that was unable to be obtained despite requests to authors.

4.6.1 Sources of Bias

Publication bias is present when research is published that does not represent the total body of research conducted. Funnel plots may be used to help identify potential publication bias. As a funnel plot is used for 10 or more studies, it was not possible to generate one for this review but the suspiscion exists that studies with negative outcomes were not accepted for publication. As the majrity of studies in this review reported positive or neutral conclusions despite small populations samples and questions over the clinical relevance of changes found. It is important to note that inspection and analysis of funnel plots should not be the only method of identifying publication and reporting bias. By following the clear JBI systematic review process this review has minimised the likelihood of bias by thoroughly and systematically searching available information sources.[307] The source of funding for publications may also introduce bias. Two studies using commercially available preparations acknowledged funding from the companies associated with the products. It is recognised that industry funded research can increase pressure for favourable reporting of outcomes. However the likelihood of funding bias applying to probiotic research for acute diarhhea has been investigated and shown not to influence positive outcomes.[308]

4.7 Looking forward

4.7.1 The future of probiotics

As the knowledge base underlying microbial interactions grows, there is the development of next generation 'bioengineered' probiotics which can be tailored create more precise, consistent and desired effect on the human immune system. For example, genetically engineered *Lactococcus lactis* strains have been designed to secrete the anti inflammatory cytkine interleukin-35, and have been shown to effectively reduce the incidence and disease severity of collagen induced arthritis in mice.[309] Much research remains at an early stage, in murine trials and benefits identified in animal studies are not always replicated in human trials.[310] However, it does provide the possibility of more targeted approaches for probiotics in the future. Whilst essential research is ongoing, members of the public continue to use the many commercially available brands of probiotics, in 2015 McFarland et al.[51] identified >100 different probiotic products available globally. Therefore, clinicians still require the capacity to discuss the general benefits and harms of current products in a transparent manner with their patients

4.7.2 Key features for future work on probiotics in IA.

Secondary research, the analysis of existing data, may no longer be appropriate as it has been conducted several times and the existing literature has too many limitations to yield further findings. Given the rate of development of new knowledge regarding specific strains, new technology to interpret the gut microbiome and new insights into individual microbial determinants further rigorous primary research to creating better data is the suggested course of action. There remain many recommendations for primary research which would aim to address the limitations of the current body of evidence by:

1. Addressing sample populations that carefully address population based confounding factors such as age, gender, disease duration,diet and body weight.

2. Investigating the ethical application of probiotics at specific windows of opportunity for reducing inflammation. This may be targeting individuals 'at risk' of inflammatory arthritis or investigating the role, if any, of probiotics in maintaining remission.

2.Employing patient stratification according to new information on permissive phenotypes as person-specific factors may drive individualised disease manifestations and responses to treatment.

3.Examining emotional and cognitive affects of probiotic consumption in rheumatology patients in order to clarify the mechanisms of effect upon quality of life and pain.

4.Repeating studies using rigorously identified probiotics species in order to identify any dose dependant responses and to allow stratification of patients by treatment responsiveness and susceptibility to adverse effects.

5. Employing validation and verification of probiotic products at multiple stages of the clinical trial through product batch and lot testing. This may be recommeded to include screening for antibiotic resistant genes.

6. Broadening research to include the application of probiotics to oral mucosal surfaces as oral mucosa are more accessible for sampling and intervention.

It has been suggested that probiotic research is fundamentally unsuited to an randomised controlled trial approach, because this pre supposes population uniformity and intervention homogeneity which, as we learn more about the interaction of genetics, lifestyle, diet and medications becomes more difficult to control through clinical trial exclusion criteria. Therefore, appropriate primary research may not be large scale randomised control trials but targeted small scale experimental trials which aim to match personal microbiomes and dysbiosis to specifically bioengineered formulations. Whilst the core OMERACT domains include the development of economic studies, robust evidence of the clinical benefit of the intervention is required before proceeding to cost benefit analysis therefore this has not been included as a key research recommendation.

4.8 Implications for clinical practice

There have been five systematic reviews now conducted investigating the outcome of probiotic supplementation for individuals with rheumatological conditions. Determining what level of evidence is sufficient to support the use, promotion or even discussion of supplements such as probiotics which are not subject to the same level of rigour in trials compared to medications continues to be debated. Knowledge continues to evolve and this will shape the strength of recommendations possible. Current international collaborations are expanding the metagenomics database for the gastrointestinal tract. This will aid the precision and accuracy of metagenomic analyses that are required to understand the

marked difference in personal microbiome and facilitate the development of personalised and predictive medicine. In the future, the individual microbiome signature for patients may be matched to probiotic regimes facilitating specific and predictable outcomes for bacterial mechanisms of action. This may then enable targeted mechanistic research and shorter translation pipelines back to clinical practise than the isolated study of every possible probiotics strain and every condition.

Despite the potential of further breakthroughs in microbiome understanding and patient stratification, the author of this thesis would still suggest that the results of this systematic review can be applied to inform patient clinician interactions and communication about the current use of probiotics in rheumatology in plain English as follows:
Current research has mainly been undertaken on older individuals, with low markers of inflammation and longstanding RA. Few studies have focused on SpA. Probiotics appear safe for this type of patient, when taken alongside most traditional medications but this has not been demonstrated alongside the newer biological DMARDS. The specific formulations, doses or durations of probiotics cannot yet be specified, and minor side effects can still occur. The studies found some small benefits that improved quality of life, reduced pain (specifically for formulations of lactobacillus for reducing pain) and lowered/maintained inflammation (specifically for mixed formulations). These small benefits may not be clinically meaningful and do not justify the use of probiotics as sole interventions for IA. In summary, there is little robust evidence to support their use for managing RA and SpA, but if you choose to consider probiotics they should be discussed with your specialist to prevent any interaction between your prescribed medications and these supplements.

References

1. Ackerman IN, Bohensky MA, Pratt C, Gorelik A, Liew D. Counting the cost. *Part 1: Healthcare Costs The current and future burden of arthritis.* 2016.
2. Cross M, Smith E, Hoy D, et al. The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis. 2014;73(7):1316-1322.
3. Briggs AM, Cross MJ, Hoy DG, et al. Musculoskeletal Health Conditions Represent a Global Threat to Healthy Aging: A Report for the 2015 World Health Organization World Report on Ageing and Health. *Gerontologist.* 2016;56 Suppl 2:S243-255.
4. Stolwijk C, van Onna M, Boonen A, van Tubergen A. Global Prevalence of Spondyloarthritis: A Systematic Review and Meta-Regression Analysis. *Arthritis Care Res.* 2016;68(9):1320-1331.
5. Michelsen B, Fiane R, Diamantopoulos AP, et al. A comparison of disease burden in rheumatoid arthritis, psoriatic arthritis and axial spondyloarthritis. *PLoS One.* 2015;10(4):e0123582.
6. Zamora NV, Christensen R, Goel N, et al. Critical Outcomes in Longitudinal Observational Studies and Registries in Patients with Rheumatoid Arthritis: An OMERACT Special Interest Group Report. *J Rheumatol.* 2017;44(12):1894-1898.
7. Kenna TJ, Hanson A, Costello M-E, Brown MA. Functional genomics and its bench-to-bedside translation pertaining to the identified susceptibility alleles and loci in ankylosing spondylitis. *Curr Rheumatol Rep.* 2016;18(10):63.
8. Catrina AI, Deane KD, Scher JU. Gene, environment, microbiome and mucosal immune tolerance in rheumatoid arthritis. *Rheumatology.* 2016;55(3):391-402.
9. Rantapää-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* 2003;48(10):2741-2749.
10. Zhang X, Zhang D, Jia H, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med.* 2015;21(8):895-905.
11. Costello ME, Ciccia F, Willner D, et al. Brief report: intestinal dysbiosis in ankylosing spondylitis. *Arthritis Rheumatol.* 2015;67(3):686-691.
12. Scarpa R, Manguso F, D'Arienzo A, et al. Microscopic inflammatory changes in colon of patients with both active psoriasis and psoriatic arthritis without bowel symptoms.*J Rheumatol.* 2000;27(5):1241-1246.
13. Barthel D, Ganser G, Kuester R-M, et al. Inflammatory bowel disease in juvenile idiopathic arthritis patients treated with biologics. *J Rheumatol*. 2015;42(11):2160-2165..
14. Stoll ML, Patel AS, Punaro M, Dempsey-Robertson M. MR enterography to evaluate sub-clinical intestinal inflammation in children with spondyloarthritis. *Pediatr Rheumatol.* 2012;10(1):6.
15. Craig E, Cappelli LC. Gastrointestinal and Hepatic Disease in Rheumatoid Arthritis. *Rheum Dis Clin North Am.* 2018;44(1):89-111.
16. Hughes CD, Pollard LC, Scott DL. 094 A Systematic Review of the Impact of Intensive Therapy on Remission in Rheumatoid Arthritis.*Rheumatology(Oxford).* 2016;55(1):i100-i100.
17. Rubbert-Roth A, Finckh A. Treatment options in patients with rheumatoid arthritis failing initial TNF inhibitor therapy: a critical review. *Arthritis Res Ther.* 2009;11(1):S1.

18. Guzman J, Oen K, Tucker LB, et al. The outcomes of juvenile idiopathic arthritis in children managed with contemporary treatments: results from the ReACCh-Out cohort. *Ann Rheum Dis.* 2015;74(10):1854-1860.
19. van der Heijde D, Kivitz A, Schiff MH, et al. Efficacy and safety of adalimumab in patients with ankylosing spondylitis: results of a multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum.* 2006;54(7):2136-2146.
20. Carron P, Varkas G, Renson T, Colman R, Elewaut D, Van den Bosch F. High Rate of Drug-Free Remission After Induction Therapy With Golimumab in Early Peripheral Spondyloarthritis. *Arthritis Rheumatol.* 2018;70(11):1769-1777.
21. Goodacre L, Goodacre J. Factors influencing the beliefs of patients with rheumatoid arthritis regarding disease-modifying medication. *Rheumatology.* 2004;43(5):583-586.
22. Segan JD, Briggs AM, Chou L, et al. Patient-perceived health service needs in inflammatory arthritis: a systematic scoping review. *Semin Arthritis Rheum* 2018 Jun 1 (Vol. 47, No. 6, pp. 765-777). WB Saunders
23. Agheyisi R. The probiotics market: Ingredients, supplements, foods. *Report code: FOD035B, BCC Research, Wellesley, MA, USA.* 2008.
24. Sharp RR, Achkar J-P, Brinich MA, Farrell RM. Helping patients make informed choices about probiotics: a need for research. *Am J Gastroenterol.* 2009;104(4):809.
25. Schultz M, Baranchi A, Thurston L, et al. Consumer demographics and expectations of probiotic therapy in New Zealand: results of a large telephone survey. *N Z Med (Online).* 2011;124(1329).
26. Sirois FM. Health-related self-perceptions over time and provider-based complementary and alternative medicine (CAM) use in people with inflammatory bowel disease or arthritis. *Complement Ther Med.* 2014;22(4):701-709.
27. Fautrel B, Adam V, St-Pierre Y, Joseph L, Clarke AE, Penrod JR. Use of complementary and alternative therapies by patients self-reporting arthritis or rheumatism: results from a nationwide canadian survey. *J Rheumatol.* 2002;29(11):2435-2441.
28. Chatfield SM, Dharmage SC, Boers A, et al. Complementary and alternative medicines in ankylosing spondylitis: a cross-sectional study. *Clin Rheumatol.* 2009;28(2):213-217.
29. Yang L, Sibbritt D, Adams J. A critical review of complementary and alternative medicine use among people with arthritis: a focus upon prevalence, cost, user profiles, motivation, decision-making, perceived benefits and communication. *Rheumatol Intl.* 2017;37(3):337-351.
30. Rao JK, Mihaliak K, Kroenke K, Bradley J, Tierney WM, Weinberger M. Use of complementary therapies for arthritis among patients of rheumatologists. *Ann Intern Med.* 1999;131(6):409-416.
31. Robinson A, McGrail M. Disclosure of CAM use to medical practitioners: a review of qualitative and quantitative studies. *Complement Ther Med.* 2004;12(2-3):90-98.
32. Danve A, Deodhar AA. Complementary medicine for axial spondyloarthritis: is there any scientific evidence? *Curr Opin Rheumatol.* 2018;30(4):310-318.
33. Hill C, Guarner F, Reid G, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastro Hepat.* 2014;11(8):506-514.
34. Panda S, Guarner F, Manichanh C. Structure and functions of the gut microbiome. *Endocr Metab Immun.* 2014;14(4):290-299.
35. Ignacio A, Morales CI, Câmara NOS, Almeida RR. Innate sensing of the gut microbiota: modulation of inflammatory and autoimmune diseases. *Front Immunol.* 2016;7:54.

36. Ghouri YA, Richards DM, Rahimi EF, Krill JT, Jelinek KA, DuPont AW. Systematic review of randomized controlled trials of probiotics, prebiotics, and synbiotics in inflammatory bowel disease. *Clin Exper Gastroenterol.* 2014;7:473.
37. Ford AC, Quigley EM, Lacy BE, et al. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. *Am J Gastroenterol.* 2014;109(10):1547.
38. Saez-Lara MJ, Gomez-Llorente C, Plaza-Diaz J, Gil A. The role of probiotic lactic acid bacteria and bifidobacteria in the prevention and treatment of inflammatory bowel disease and other related diseases: a systematic review of randomized human clinical trials. *BioMed Res Int.* 2015;2015.
39. Scher JU, Littman DR, Abramson SB. Microbiome in inflammatory arthritis and human rheumatic diseases. *Arthritis Rheumatol.* 2016;68(1):35-45.
40. Wang P, Tao J-H, Pan H-F. Probiotic bacteria: a viable adjuvant therapy for relieving symptoms of rheumatoid arthritis. *Inflammopharmacology.* 2016;24(5):189-196.
41. Derwa Y, Gracie D, Hamlin P, Ford A. Systematic review with meta-analysis: the efficacy of probiotics in inflammatory bowel disease. *Aliment Pharm Ther.* 2017;46(4):389-400.
42. Amdekar S, Roy P, Singh V, Kumar A, Singh R, Sharma P. Anti-inflammatory activity of lactobacillus on carrageenan-induced paw edema in male wistar rats. *Int J Inflamm.* 2012;2012.
43. Snydman DR. The safety of probiotics. *Clin Infect Dis.* 2008;46(Supplement_2):S104-S111.
44. Didari T, Solki S, Mozaffari S, Nikfar S, Abdollahi M. A systematic review of the safety of probiotics. *Expert Opin Drug Saf.* 2014;13(2):227-239.
45. Bongaerts GP, Severijnen RS. A reassessment of the PROPATRIA study and its implications for probiotic therapy. *Nature Biotech.* 2016;34(1):55.
46. Chin-Lee B, Curry WJ, Fetterman J, Graybill MA, Karpa K. Patient experience and use of probiotics in community-based health care settings. *Patient Prefer Adher.* 2014;8:1513.
47. Hamilton-Miller J, Gibson GR, Bruck W. Some insights into the derivation and early uses of the word 'probiotic'. *Brit J Nutr.* 2003;90(4):845-845.
48. Chilton SN, Burton JP, Reid G. Inclusion of fermented foods in food guides around the world. *Nutrients.* 2015;7(1):390-404.
49. Gogineni VK, Morrow LE, Gregory PJ, Malesker MA. Probiotics: history and evolution. *J Ancient Dis Prev Rem.* 2013.
50. Anukam KC, Reid G. Probiotics: 100 years (1907-2007) after Elie Metchnikoff's observation. *Communicating current research and educational topics and trends in applied microbiology.* 2007 Ed Mendez-Vilas Formatex, Spain;1:466-474.
51. McFarland LV. From Yaks to Yogurt: The History, Development, and Current Use of Probiotics. *Clin Infect Dis.* 2015;60(suppl_2):S85-S90.
52. Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. *International journal of environmental research and public health.* 2014;11(5):4745-4767.
53. Frank DN PN. Gastrointestinal microbiology enters the metagenomics era. *Curr Opin Gastroenterol.* 2008;24(1):4-10.
54. Aagaard K PJ, Keitel W, Watson M, et al. The Human Microbiome Project strategy for comprehensive sampling of the human microbiome and why it matters. *FASEB J.* 2013;27(3):1012-1022.
55. Zou S CL, Colombini-Hatch S, Glynn S, Srinivas P. Research on the human virome: where are we and what is next. *Microbiome.* 2016;4(1):32.

56. Stulberg E FD, Proctor LM, Murray DM, et al An assessment of US microbiome research. *Nature Microbiol.* 2016;1(1):150-151.
57. Norman JM HS, Baldridge MT, Droit L, et al. Disease-specific alterations in the enteric virome in inflammatory bowel disease. *Cell.* 2015;160(3):447-460.
58. Abeles SR PD. Molecular bases and role of viruses in the human microbiome. *J Mol Biol.* 2014;426(23):3892-3906.
59. Yarza P YP, Pruesse E, Glöckner FO, et al. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 2014;12(9):635.
60. Ciccarelli FD DT, Von Mering C, Creevey CJ, Snel B, Bork P. Toward automatic reconstruction of a highly resolved tree of life. *Science.* 2006;311(5765):1283-1287.
61. Letunic I BP. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics.* 2006;23(1):127-128.
62. Bull MJ, Jolley KA, Bray JE, et al. The domestication of the probiotic bacterium Lactobacillus acidophilus. *Sci Rep.* 2014;4:7202.
63. Douillard FP, Mora D, Eijlander RT, Wels M, De Vos WM. Comparative genomic analysis of the multispecies probiotic-marketed product VSL# 3. *PloS one.* 2018;13(2):e0192452.
64. Sanders ME, Benson A, Lebeer S, Merenstein DJ, Klaenhammer TR. Shared mechanisms among probiotic taxa: implications for general probiotic claims. *Curr Opin Biotech.* 2018;49:207-216.
65. Johnson EA. Biotechnology of non-Saccharomyces yeasts—the ascomycetes. *App Microbiol Biotech.* 2013;97(2):503-517.
66. Rima H, Steve L, Ismail F. Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. *Front Microbiol.* 2012;3:421.
67. Marquina D, Santos A, Peinado J. Biology of killer yeasts. *Intl Microbiol.* 2002;5(2):65-71.
68. Goerges S, Aigner U, Silakowski B, Scherer S. Inhibition of Listeria monocytogenes by food-borne yeasts. *Appl Environ Microb.* 2006;72(1):313-318.
69. Van den Nieuwboer M, Van De Burgwal L, Claassen E. A quantitative key-opinion-leader analysis of innovation barriers in probiotic research and development: valorisation and improving the tech transfer cycle. *PharmaNutrition.* 2016;4(1):9-18.
70. Szajewska H, Kolodziej M. Systematic review with meta-analysis: Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. *Aliment Pharmacol Ther.* 2015;42(7):793-801.
71. Dalmasso G, Cottrez F, Imbert V, et al. Saccharomyces boulardii inhibits inflammatory bowel disease by trapping T cells in mesenteric lymph nodes. *Gastroenterology.* 2006;131(6):1812-1825.
72. Vitetta L, Coulson S, Thomsen M, Nguyen T, Hall S. Probiotics, D–Lactic acidosis, oxidative stress and strain specificity. *Gut microbes.* 2017;8(4):311-322.
73. Makras L, Triantafyllou V, Fayol-Messaoudi D, et al. Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards Salmonella enterica serovar Typhimurium reveals a role for lactic acid and other inhibitory compounds. *Res Microbiol.* 2006;157(3):241-247.
74. Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. Probiotic Mechanisms of Action. *Ann Nutr Metab.* 2012;61(2):160-174.
75. Karczewski J, Troost FJ, Konings I, et al. Regulation of human epithelial tight junction proteins by Lactobacillus plantarum in vivo and protective effects on the epithelial barrier. *Am J Physiol Gastrointest Liver Physiol.* 2010;298(6):G851-G859.

76. Lebeer S, Bron PA, Marco ML, et al. Identification of probiotic effector molecules: present state and future perspectives. *Curr Opin Biotech.* 2018;49:217-223.
77. Dwivedi M, Kumar P, Laddha NC, Kemp EH. Induction of regulatory T cells: a role for probiotics and prebiotics to suppress autoimmunity. *Autoimmun Rev.* 2016;15(4):379-392.
78. Vighi G, Marcucci F, Sensi L, Di Cara G, Frati F. Allergy and the gastrointestinal system. *Clin Exp Immunol.* 2008;153:3-6.
79. Begley M, Hill C, Gahan CG. Bile salt hydrolase activity in probiotics. *Appl Environ Microbiol.* 2006;72(3):1729-1738.
80. Gu Q, Li P. Biosynthesis of Vitamins by Probiotic Bacteria. In: *Probiotics and Prebiotics in Human Nutrition and Health.* InTech; 2016.
81. Rossi M, Amaretti A, Raimondi S. Folate production by probiotic bacteria. *Nutrients.* 2011;3(1):118-134.
82. Whittle S, Hughes R. Folate supplementation and methotrexate treatment in rheumatoid arthritis: a review. *Rheumatology.* 2004;43(3):267-271.
83. Sanders ME, Akkermans LM, Haller D, et al. Safety assessment of probiotics for human use. *Gut Microbes.* 2010;1(3):164-185.
84. WHO. *Guidelines for the evaluation of probiotics in food: report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food,* London Ontario, Canada, April 30 and May 1, 2002 2002.
85. WHO. *Laboratory Biosafety Manual.* World Health Organization; 2004.
86. Vankerckhoven V, Huys G, Vancanneyt M, et al. Genotypic diversity, antimicrobial resistance, and virulence factors of human isolates and probiotic cultures constituting two intraspecific groups of Enterococcus faecium isolates. *Appl Environ Microbiol.* 2008;74(14):4247-4255.
87. Imperial IC, Ibana JA. Addressing the Antibiotic Resistance Problem with Probiotics: Reducing the Risk of Its Double-Edged Sword Effect. *Front Microbiol.* 2016;7:1983.
88. Hu Y, Yang X, Qin J, et al. Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. *Nature Comms.* 2013;4:2151.
89. Mercer M BM, Geller G, Harrison K, et al. How patients view probiotics: findings from a multicenter study of patients with inflammatory bowel disease and irritable bowel syndrome. *J Clin Gastroenterol.* 2012;46(2):138.
90. Boers M, Kirwan JR, Wells G, et al. Developing core outcome measurement sets for clinical trials: OMERACT filter 2.0. *J Clin Epidemiol.* 2014;67(7):745-753.
91. Boers M, Tugwell P. OMERACT conference questionnaire results. OMERACT Committee. *J Rheumatol.* 1993;20(3):552-554.
92. National Institutes of Health NCI. Common terminology criteria for adverse events (CTCAE) . . In: Services. UDoHaH, ed. Vol 28. version 4.0 ed2009:3.Accessed online Oct 2018 https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm
93. Lassere MN, Johnson KR, Boers M, et al. Standardized assessment of adverse events in rheumatology clinical trials: summary of the OMERACT 7 drug safety module update. *J Rheumatol.* 2005;32(10):2037-2041.
94. Coulter A. Measuring what matters to patients. *BMJ.* 2017;356:j816.
95. Felson DT, Anderson JJ, Boers M, et al. The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. The Committee on Outcome Measures in Rheumatoid Arthritis Clinical Trials. *Arthritis Rheum.* 1993;36(6):729-740.

96. De Gruttola VG, Clax P, DeMets DL, et al. Considerations in the evaluation of surrogate endpoints in clinical trials. summary of a National Institutes of Health workshop. *Control Clin Trials.* 2001;22(5):485-502.
97. Siemons L, Den Klooster PM, Vonkeman HE, van Riel PL GC, MA. vdL. How Age, Gender and Body Mass Index Affect the Erythrocyte Sedimentation Rate and the C-Reactive Protein in Early Rheumatoid Arthritis *Ann Rheum Dis.* 2014;1(73):387.
98. Nielung L, Christensen R, Danneskiold-Samsoe B, et al. Validity and Agreement between the 28-Joint Disease Activity Score Based on C-Reactive Protein and Erythrocyte Sedimentation Rate in Patients with Rheumatoid Arthritis. *Arthritis.* 2015;2015:401690.
99. Crowson CS, Rahman MU, Matteson EL. Which measure of inflammation to use? A comparison of erythrocyte sedimentation rate and C-reactive protein measurements from randomized clinical trials of golimumab in rheumatoid arthritis. *J Rheumatol.* 2009;36(8):1606-1610.
100. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* 2014;505(7484):559-563.
101. Burska A, Boissinot M, Ponchel F. Cytokines as biomarkers in rheumatoid arthritis. *Mediators Inflamm.* 2014;2014:545493.
102. Robinson WH, Mao R. Biomarkers to guide clinical therapeutics in rheumatology? *Curr Opin Rheumatol.* 2016;28(2):168-175.
103. Silveira-Nunes G, Speziali E, Teixeira-Carvalho A, et al. Lifewide profile of cytokine production by innate and adaptive immune cells from Brazilian individuals. *Immun Ageing.* 2017;14:2.
104. Goetzl EJ, Huang MC, Kon J, et al. Gender specificity of altered human immune cytokine profiles in aging. *FASEB J.* 2010;24(9):3580-3589.
105. Chen CH, Chen HA, Liao HT, Liu CH, Tsai CY, Chou CT. Suppressors of cytokine signalling in ankylosing spondylitis and their associations with disease severity, acute-phase reactants and serum cytokines. *Clin Exp Rheumatol.* 2016;34(1):100-105.
106. Shrivastava AK, Singh HV, Raizada A, et al. Inflammatory markers in patients with rheumatoid arthritis. *Allergol Immunopathol (Madr).* 2015;43(1):81-87.
107. Scott DL, Antoni C, Choy EH, Van Riel PC. Joint counts in routine practice. *Rheumatology.* 2003;42(8):919-923.
108. Cheung PP, Gossec L, Mak A, March L. Reliability of joint count assessment in rheumatoid arthritis: a systematic literature review. *Semin Arthritis Rheum.* 2014;43(6):721-729.
109. Van der Heide DM, M VH, V VRPL, de Puttle. Development of a disease activity score based on judgment in clinical practice by rheumatologists. *J Rheumatology.* 1993;20:579-581.
110. Salaffi F, Cimmino MA, Leardini G, Gasparini S, Grassi W. Disease activity assessment of rheumatoid arthritis in daily practice: validity, internal consistency, reliability and congruency of the Disease Activity Score including 28 joints (DAS28) compared with the Clinical Disease Activity Index (CDAI). *Clin Exp Rheumatol.* 2009;27(4):552-559.
111. Uhlig T, Kvien TK, Pincus T. Test-retest reliability of disease activity core set measures and indices in rheumatoid arthritis. *Ann Rheum Dis.* 2009;68(6):972-975.
112. Leeb BF, Andel I, Sautner J, et al. Disease activity measurement of rheumatoid arthritis: Comparison of the simplified disease activity index (SDAI) and the disease activity score including 28 joints (DAS28) in daily routine. *Arthritis Rheum.* 2005;53(1):56-60.
113. Ranganath VK, Yoon J, Khanna D, et al. Comparison of composite measures of disease activity in an early seropositive rheumatoid arthritis cohort. *Ann Rheum Dis.* 2007;66(12):1633-1640.

114. Pincus T, Strand V, Koch G, et al. An index of the three core data set patient questionnaire measures distinguishes efficacy of active treatment from that of placebo as effectively as the American College of Rheumatology 20% response criteria (ACR20) or the Disease Activity Score (DAS) in a rheumatoid arthritis clinical trial. *Arthritis Rheum.* 2003;48(3):625-630.
115. Glinatsi D, Baker JF, Hetland ML, et al. Magnetic resonance imaging assessed inflammation in the wrist is associated with patient-reported physical impairment, global assessment of disease activity and pain in early rheumatoid arthritis: longitudinal results from two randomised controlled trials. *Ann Rheum Dis.* 2017;76(10):1707-1715.
116. Merskey H, Bogduk N. Classification of Chronic Pain, Second Edition, IASP Task Force on Taxonomy IASP Task Force on Taxonomy. *Seattle, "Part III: Pain Terms, A Current List with Definitions and Notes on Usage" (pp 209-214) edited by H. Merskey and N. Bogduk, IASP Press, Seattle, ©1994*.Accessed online https://s3.amazonaws.com/rdcms-iasp/files/production/public/Content/ContentFolders/Publications2/FreeBooks/Classification-of-Chronic-Pain.pdf Oct 2018
117. Kidd BL, Urban LA. Mechanisms of inflammatory pain. *Br J Anaesth.* 2001;87(1):3-11.
118. Nazemian V, Shadnoush M, Manaheji H, J Z. Probiotics and inflammatory pain: a literature review study. *Middle East J Rehab Health.* 2016;3(2).
119. Fereshteh Dardmeh, Hans Ingolf Nielsen, Hiva Alipour, Benedict Kjærgaard, Erik Brandsborg, Gazeran P. Potential Nociceptive Regulatory Effect of Probiotic Lactobacillus rhamnosus PB01 (DSM 14870) on Mechanical Sensitivity in Diet-Induced Obesity Model. *Pain Res Manag.* 2016:7.
120. Carabottia M, Sciroccoa A, Masellib MA. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol.* 2015;28(1):1-7.
121. Saulnier DM, Ringel Y, Heyman MB, et al. The intestinal microbiome, probiotics and prebiotics in neurogastroenterology. *Gut Microbes.* 2013;4(1):17-27.
122. Distrutti E, Cipriani S, Mencarelli A, Renga B, Fiorucci S. Probiotics VSL#3 protect against development of visceral pain in murine model of irritable bowel syndrome. *PLoS One.* 2013;8(5):e63893.
123. Fitzcharles MA, Shir Y. Management of chronic pain in the rheumatic diseases with insights for the clinician. *Ther Adv Musculoskelet Dis.* 2011;3(4):179-190.
124. Jaeschke R, Singer J, Guyatt GH. Measurement of health status. Ascertaining the minimal clinically important difference. *Control Clin Trials.* 1989;10(4):407-415.
125. Stauffer ME, Taylor SD, Watson DJ, Peloso PM, Morrison A. Definition of nonresponse to analgesic treatment of arthritic pain: an analytical literature review of the smallest detectable difference, the minimal detectable change, and the minimal clinically important difference on the pain visual analog scale. *Int J Inflam.* 2011;2011:231926.
126. Clark P, Lavielle P, Martinez H. Learning from pain scales: patient perspective. *J Rheumatol.* 2003;30(7):1584-1588.
127. Hawker GA, Mian S, Kendzerska T, French M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). *Arthritis Care Res (Hoboken).* 2011;63 Suppl 11:S240-252.
128. Brown GA. AAOS clinical practice guideline: treatment of osteoarthritis of the knee: evidence-based guideline, 2nd edition. *J Am Acad Orthop Surg.* 2013;21(9):577-579.

129. Soanes C, Elliot J. *Oxford English Dictionary.* Oxford, UK: Oxford University press; 2005.Accessed online https://www.oed.com/ Sept 2017
130. Overman CL, Kool MB, Da Silva JA, Geenen R. The prevalence of severe fatigue in rheumatic diseases: an international study. *Clin Rheumatol.* 2016;35(2):409-415.
131. Ream E, Richardson A. Fatigue: a concept analysis. *Int J Nurs Stud.* 1996;33(5):519-529.
132. van der Heijde D, Calin A, Dougados M, Khan MA, van der Linden S, Bellamy N. Selection of instruments in the core set for DC-ART, SMARD, physical therapy, and clinical record keeping in ankylosing spondylitis. Progress report of the ASAS Working Group. Assessments in Ankylosing Spondylitis. *J Rheumatol.* 1999;26(4):951-954.
133. Orbai AM, de Wit M, Mease PJ, et al. Updating the Psoriatic Arthritis (PsA) Core Domain Set: A Report from the PsA Workshop at OMERACT 2016. *J Rheumatol.* 2017;44(10):1522-1528.
134. Kirwan JR, Minnock P, Adebajo A, et al. Patient perspective: fatigue as a recommended patient centered outcome measure in rheumatoid arthritis. *J Rheumatol.* 2007;34(5):1174-1177.
135. Belza B, Miyawaki C, Liu M, Zhang X, M. F. The Multidimensional Assessment of Fatigue Scale: A 25-year Review and Evaluation. *Arthritis Rheumatol.* 2015;1(67):3077-3078.
136. Druce KL, Bhattacharya Y, Jones GT, Macfarlane GJ, Basu N. Most patients who reach disease remission following anti-TNF therapy continue to report fatigue: results from the British Society for Rheumatology Biologics Register for Rheumatoid Arthritis. *Rheumatology (Oxford).* 2016;55(10):1786-1790.
137. Dittner AJ, Wessely SC, Brown RG. The assessment of fatigue: a practical guide for clinicians and researchers. *J Psychosom Res.* 2004;56(2):157-170.
138. LaChapelle DL, Finlayson MA. An evaluation of subjective and objective measures of fatigue in patients with brain injury and healthy controls. *Brain Inj.* 1998;12(8):649-659.
139. van Steenbergen HW, Tsonaka R, Huizinga TW, Boonen A, van der Helm-van Mil AH. Fatigue in rheumatoid arthritis; a persistent problem: a large longitudinal study. *RMD Open.* 2015;1(1):e000041.
140. Dernis-Labous E, Messow M, Dougados M. Assessment of fatigue in the management of patients with ankylosing spondylitis. *Rheumatology (Oxford).* 2003;42(12):1523-1528.
141. Wolfe F. Fatigue assessments in rheumatoid arthritis: comparative performance of visual analog scales and longer fatigue questionnaires in 7760 patients. *J Rheumatol.* 2004;31(10):1896-1902.
142. Jutley GS, Sahbudin I, Nightingale P, et al. Fatigue in patients with early inflammatory arthritis and clinically suspect arthralgia patients. *Rheumatology (Oxford).* 2017 1(56).
143. Nikolaus S, Bode C, Taal E, MA. vdL. Fatigue and factors related to fatigue in rheumatoid arthritis: a systematic review. *Arthrit care Res.* 2013 65(7):1128-1146.
144. Pollard LC , Choy EH , Gonzalez J , Khoshaba B , DL S. Fatigue in rheumatoid arthritis reflects pain, not disease activity *Rheumatology (Oxford).* 2006 45(7):885-889.
145. Hewlett S, Dures E, Almeida C. Measures of fatigue: Bristol Rheumatoid Arthritis Fatigue Multi-Dimensional Questionnaire (BRAF MDQ), Bristol Rheumatoid Arthritis Fatigue Numerical Rating Scales (BRAF NRS) for severity, effect, and coping, Chalder Fatigue Questionnaire (CFQ), Checklist Individual Strength (CIS20R and CIS8R), Fatigue Severity Scale (FSS), Functional Assessment Chronic Illness Therapy (Fatigue) (FACIT-F), Multi-Dimensional Assessment of Fatigue (MAF), Multi-Dimensional Fatigue Inventory (MFI), Pediatric Quality Of Life (PedsQL) Multi-Dimensional Fatigue Scale, Profile of Fatigue (ProF), Short Form 36 Vitality Subscale (SF-36 VT), and Visual Analog Scales (VAS). *Arthrit Care R.* 2011;63 Suppl 11:S263-286.

146. Dieterich W, Schink M, Zopf Y. Microbiota in the Gastrointestinal Tract. *Med Sci (Basel).* 2018;6(4):116.
147. Stebbings S, Jenks K, Treharne GJ, et al. Validation of the Dudley Inflammatory Bowel Symptom Questionnaire for the assessment of bowel symptoms in axial SpA: prevalence of clinically relevant bowel symptoms and association with disease activity. *Rheumatology (Oxford).* 2012;51(5):858-865.
148. Bengtsson M, Ohlsson B, Ulander K. Development and psychometric testing of the Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS). *BMC Gastroenterol.* 2007;7:16.
149. Dimenas E, Carlsson G, Glise H, Israelsson B, Wiklund I. Relevance of norm values as part of the documentation of quality of life instruments for use in upper gastrointestinal disease. *Scand J Gastroenterol Suppl.* 1996;221:8-13.
150. Kulich KR, Madisch A, Pacini F, et al. Reliability and validity of the Gastrointestinal Symptom Rating Scale (GSRS) and Quality of Life in Reflux and Dyspepsia (QOLRAD) questionnaire in dyspepsia: a six-country study. *Health Qual Life Outcomes.* 2008;6:12.
151. Brunner HI, Johnson AL, Barron AC, et al. Gastrointestinal symptoms and their association with health-related quality of life of children with juvenile rheumatoid arthritis: validation of a gastrointestinal symptom questionnaire. *J Clin Rheumatol.* 2005;11(4):194-204.
152. Png K, Kwan YH, Leung YY, et al. Measurement properties of patient reported outcome measures for spondyloarthritis: A systematic review. *Semin Arthritis Rheum.* 2018;48(2):274-282.
153. Anderson JK, Zimmerman L, Caplan L, Michaud K. Measures of rheumatoid arthritis disease activity: Patient (PtGA) and Provider (PrGA) Global Assessment of Disease Activity, Disease Activity Score (DAS) and Disease Activity Score with 28-Joint Counts (DAS28), Simplified Disease Activity Index (SDAI), Clinical Disease Activity Index (CDAI), Patient Activity Score (PAS) and Patient Activity Score-II (PASII), Routine Assessment of Patient Index Data (RAPID), Rheumatoid Arthritis Disease Activity Index (RADAI) and Rheumatoid Arthritis Disease Activity Index-5 (RADAI-5), Chronic Arthritis Systemic Index (CASI), Patient-Based Disease Activity Score With ESR (PDAS1) and Patient-Based Disease Activity Score without ESR (PDAS2), and Mean Overall Index for Rheumatoid Arthritis (MOI-RA). *Arthritis Care Res.* 2011;63 Suppl 11:S14-36.
154. Cutolo M, Straub RH, Buttgereit F. Circadian rhythms of nocturnal hormones in rheumatoid arthritis: translation from bench to bedside. *Ann Rheum Dis.* 2008;67(7):905-908.
155. Halls S, Dures E, Kirwan J, et al. Stiffness is more than just duration and severity: a qualitative exploration in people with rheumatoid arthritis. *Rheumatology (Oxford).* 2015;54(4):615-622.
156. Orbai AM, Smith KC, S B, Leon E, Bingham. "Stiffness has different meanings, I think, to everyone": examining stiffness from the perspective of people living with rheumatoid arthritis. *Arthrit Care Res.* 2014;1(11):1662-1672.
157. Bacci ED, DeLozier AM, Lin CY, et al. Psychometric properties of morning joint stiffness duration and severity measures in patients with moderately to severely active rheumatoid arthritis. *Health Qual Life Outcomes.* 2017;15(1):239.
158. K M, F B, R. T. Impact of morning stiffness on working behaviour and performance in people with rheumatoid arthritis. *Rheumatol Int.* 2014;34:1751-1758.
159. Kilic L, Erden A, Bingham CO, 3rd, Gossec L, Kalyoncu U. The Reporting of Patient-reported Outcomes in Studies of Patients with Rheumatoid Arthritis: A Systematic Review of 250 Articles. *J Rheumatol.* 2016;43(7):1300-1305.

160. Woolacott NF, Corbett MS, Rice SJ. The use and reporting of WOMAC in the assessment of the benefit of physical therapies for the pain of osteoarthritis of the knee: findings from a systematic review of clinical trials. *Rheumatology (Oxford).* 2012;51(8):1440-1446.
161. Nikiphorou E, Radner H, Chatzidionysiou K,, et al. Patient global assessment in measuring disease activity in rheumatoid arthritis: a review of the literature. *Arthrit Res Ther* 2016;18:251.
162. Meenan RF, Gertman PM, Mason JH. Measuring health status in arthritis. The arthritis impact measurement scales. *Arthritis Rheum.* 1980;23(2):146-152.
163. Angst F, Aeschlimann A, Stucki G. Smallest detectable and minimal clinically important differences of rehabilitation intervention with their implications for required sample sizes using WOMAC and SF-36 quality of life measurement instruments in patients with osteoarthritis of the lower extremities. *Arthritis Rheum.* 2001;45(4):384-391.
164. Navarro-Sarabia F, Ruiz-Montesinos D, Hernandez B, et al. DAS-28-based EULAR response and HAQ improvement in rheumatoid arthritis patients switching between TNF antagonists. *BMC Musculoskelet Disord.* 2009;10:91.
165. Kwan YH, Fong W, Lui NL, et al. Validity and reliability of the Health Assessment Questionnaire among patients with spondyloarthritis in Singapore. *Int J Rheum Dis.* 2018;21(3):699-704.
166. McConnell S, Kolopack P, Davis AM. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC): a review of its utility and measurement properties. *Arthritis Rheum.* 2001;45(5):453-461.
167. A C. Adult measures of quality of life: The Arthritis impact measurement scales (AIMS/AIMS2), disease repercussion Profile (DRP), EuroQoL, Nottingham health Profile (NHP), patient generated index (PGI), quality of well-being scale (QWB), RAQoL, short form-36 (SF-36), sickness impact Profile (SIP), SIP-RA, and World Health Organization's quality of life instruments (WHOQoL, WHOQoL-100, WHOQoL-Bref). *Arthrit Care Res.* 2003 49:S113-133.
168. Bellamy N, Wilson C, Hendrikz J. Population-based normative values for the Western Ontario and McMaster (WOMAC) Osteoarthritis Index: part I. *Semin Arthritis Rheum.* 2011;41(2):139-148.
169. Wells GA, Boers M, Shea B, et al. Minimal disease activity for rheumatoid arthritis: a preliminary definition. *J Rheumatol.* 2005;32(10):2016-2024.
170. Krishnan E, Sokka T, Hakkinen A, Hubert H, Hannonen P. Normative values for the Health Assessment Questionnaire disability index: benchmarking disability in the general population. *Arthritis Rheum.* 2004;50(3):953-960.
171. Challa DNV, Crowson CS, Davis JM, 3rd. The Patient Global Assessment of Disease Activity in Rheumatoid Arthritis: Identification of Underlying Latent Factors. *Rheumatol Ther.* 2017;4(1):201-208.
172. Jones SD, Steiner A, Garrett SL, Calin A. The Bath Ankylosing Spondylitis Patient Global Score (BAS-G). *Br J Rheumatol.* 1996;35(1):66-71.
173. Bruce B, Fries JF. The Health Assessment Questionnaire (HAQ). *Clin Exp Rheumatol.* 2005;23(5 Suppl 39):S14-18.
174. French T, Hewlett S, Kirwan J, Sanderson T. Different Wording of the Patient Global Visual Analogue Scale (PG-VAS) Affects Rheumatoid Arthritis Patients' Scoring and the Overall Disease Activity Score (DAS28): A Cross-Sectional Study. *Musculoskeletal care.* 2013;11(4):229-237.

175. Meenan RF, Mason JH, Anderson JJ, Guccione AA, Kazis LE. AIMS2. The content and properties of a revised and expanded Arthritis Impact Measurement Scales Health Status Questionnaire. *Arthritis Rheum.* 1992;35(1):1-10.
176. Uhlig T, Haavardsholm EA, Kvien TK. Comparison of the Health Assessment Questionnaire (HAQ) and the modified HAQ (MHAQ) in patients with rheumatoid arthritis. *Rheumatology.* 2005;45(4):454-458.
177. Khan NA, Spencer HJ, Abda EA, et al. Patient's global assessment of disease activity and patient's assessment of general health for rheumatoid arthritis activity assessment: are they equivalent? *Ann Rheum Dis.* 2012;71(12):1942-1949.
178. Markenson JA, Koenig AS, Feng JY, et al. Comparison of physician and patient global assessments over time in patients with rheumatoid arthritis: a retrospective analysis from the RADIUS cohort. *J Clin Rheumatol.* 2013;19(6):317-323.
179. Leeb BF, Sautner J, Leeb BA, Fassl C, Rintelen B. Lack of agreement between patients' and physicians' perspectives of rheumatoid arthritis disease activity changes. *Scand J Rheumatol.* 2006;35(6):441-446.
180. Irons K, Harrison H, A T, J M. *An updated synopsis of the Bath Indices – outcome measures for use with Ankylosing Spondylitis patients and their broader application.* London, W6 0QT4: National Ankylosing Spondylitis Society 2016.
181. Doward LC, Spoorenberg A, Cook SA, et al. Development of the ASQoL: a quality of life instrument specific to ankylosing spondylitis. *Ann Rheum Dis.* 2003;62(1):20-26.
182. Kviatkovsky MJ, Ramiro S, Landewe R, et al. The Minimum Clinically Important Improvement and Patient-acceptable Symptom State in the BASDAI and BASFI for Patients with Ankylosing Spondylitis. *J Rheumatol.* 2016;43(9):1680-1686.
183. Zochling J. Measures of symptoms and disease status in ankylosing spondylitis: Ankylosing Spondylitis Disease Activity Score (ASDAS), Ankylosing Spondylitis Quality of Life Scale (ASQoL), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Global Score (BAS-G), Bath Ankylosing Spondylitis Metrology Index (BASMI), Dougados Functional Index (DFI), and Health Assessment Questionnaire for the Spondylarthropathies (HAQ-S). *Arthritis Care Res.* 2011;63 Suppl 11:S47-58.
184. Calin A, Garrett S, Whitelock H, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. *J Rheumatol.* 1994;21(12):2281-2285.
185. van Tubergen A, Black PM, Coteur G. Are patient-reported outcome instruments for ankylosing spondylitis fit for purpose for the axial spondyloarthritis patient? A qualitative and psychometric analysis. *Rheumatology (Oxford).* 2015;54(10):1842-1851.
186. Clinical safety data management: definitions and standards for expedited reporting. . International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 27 October 1994.accessed online http://fercsl.lk/wp/wp-content/uploads/2019/04/Clinical-Safety-Data-Management-Definitions-Standards-for-Expedited-Reporting-ICH-Harmonised-Tripartite-Guideline-1994.pdf November 2018
187. Madsen OR. Stability of fatigue, pain, patient global assessment and the Bath Ankylosing Spondylitis Functional Index (BASFI) in spondyloarthropathy patients with stable disease according to the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). *Rheumatol Int.* 2018;38(3):425-432.
188. Pavy S, Brophy S, Calin A. Establishment of the minimum clinically important difference for the bath ankylosing spondylitis indices: a prospective study. *J Rheumatol.* 2005;32(1):80-85.

189. Ariza-Ariza R, Hernandez-Cruz B, Navarro-Compan V, Leyva Pardo C, Juanola X, Navarro-Sarabia F. A comparison of telephone and paper self-completed questionnaires of main patient-related outcome measures in patients with ankylosing spondylitis and psoriatic arthritis. *Rheumatol Int.* 2013;33(11):2731-2736.
190. *Health care costs: the future burden of arthritis* Arthritis Australia; May 2016.
191. Sackett DL, Rosenberg WM, Gray JA, Haynes RB, Richardson WS. Evidence based medicine: what it is and what it isn't. *BMJ.* 1996;312(7023):71-72.
192. Chevret S, Ferguson ND, Bellomo R. Are systematic reviews and meta-analyses still useful research? No. *Intensive Care Med.* 2018;44(4):515-517.
193. Annane D, Jaeschke R, Guyatt G. Are systematic reviews and meta-analyses still useful research? Yes. *Intensive Care Med.* 2018;44(4):512-514.
194. Aromataris E, Pearson A. The systematic review: an overview. *Am J Nurs.* 2014;114(3):53-58.
195. Guyatt G, Cairns J, Churchill D, et al. Evidence-based medicine: a new approach to teaching the practice of medicine. *Jama.* 1992;4(17):2420-2425.
196. Moller MH, Ioannidis JPA, Darmon M. Are systematic reviews and meta-analyses still useful research? We are not sure. *Intensive Care Med.* 2018;44(4):518-520.
197. Peters M, Godfrey C, McInerney P, Soares C, Khalil H, D. P. The Joanna Briggs Institute reviewers' manual 2015: methodology for JBI scoping reviews. In: Adelaide: The Joanna Briggs Institute; 2015: http://joannabriggs.org/assets/docs/sumari/Reviewers-Manual_Methodology-for-JBI-Scoping-Reviews_2015_v2.pdf.accessed Sept 2017
198. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev.* 2015;4:1.
199. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ.* 2008;336(7650):924-926.
200. Guyatt GH, Oxman AD, Schunemann HJ, Tugwell P, Knottnerus A. GRADE guidelines: a new series of articles in the Journal of Clinical Epidemiology. *J Clin Epidemiol.* 2011;64(4):380-382.
201. Jordan Z, Lockwood C, Munn Z, E. A. Redeveloping the JBI Model of Evidence Based Healthcare. *Int J Evid-based Healthc.* 2018 Dec 1;16(4):227-41
202. Pan H, Li R, Li T, Wang J, L. L. Whether probiotic supplementation benefits rheumatoid arthritis patients: a systematic review and meta-analysis.*Engineering.* 2017;3:115-121.
203. Rudbane SMA, Rahmdel S, Abdollahzadeh SM, Zare M, Bazrafshan A, Mazloomi SM. The efficacy of probiotic supplementation in rheumatoid arthritis: a meta-analysis of randomized, controlled trials. *Inflammopharmacology.* 2018;26(1):67-76.
204. Mohammed AT, Khattab M, Ahmed AM, et al. The therapeutic effect of probiotics on rheumatoid arthritis: a systematic review and meta-analysis of randomized control trials. *Clin Rheumatol.* 2017;36(12):2697-2707.
205. Dejoras EMM, Remalante PPM, ATM. S. Probiotic supplementation and its effect on disease activity in rheumatoid arthritis: A systematic review and meta-analysis. *Int J Rheum Dis.* 2017;20.
206. Mazidi M, Rezaie P, Ferns GA, Vatanparast H. Impact of Probiotic Administration on Serum C-Reactive Protein Concentrations: Systematic Review and Meta-Analysis of Randomized Control Trials. *Nutrients.* 2017;9(1).
207. Patel SJ, Kemper KJ, Kitzmiller JP. Physician perspectives on education, training, and implementation of complementary and alternative medicine. *Adv Med Educ Pract.* 2017;8:499-503.

208. Maha N, Shaw A. Academic doctors' views of complementary and alternative medicine (CAM) and its role within the NHS: an exploratory qualitative study. *BMC Complement Altern Med.* 2007;7:17-17.
209. McFarland LV, Evans CT, Goldstein EJC. Strain-Specificity and Disease-Specificity of Probiotic Efficacy: A Systematic Review and Meta-Analysis. *Front Med (Lausanne).* 2018;5:124.
210. Glanville J, King S, Guarner F, Hill C, Sanders ME. A review of the systematic review process and its applicability for use in evaluating evidence for health claims on probiotic foods in the European Union. *Nutr J.* 2015;14:16.
211. Hong HA, Duc le H, Cutting SM. The use of bacterial spore formers as probiotics. *FEMS Microbiol Rev.* 2005;29(4):813-835.
212. Lowe J., Briggs A, Whittle S,Hoon E, Stephenson M. Effectiveness of probiotics in the management of inflammatory arthritis: a systematic review protocol. *JBI Database System Rev Implement Rep* 2018:16(12)2295-2303.
. *Int J Food Microbiol.* 2010;138(3):223-231.
213. Tugwell P, Boers M. OMERACT conference on outcome measures in rheumatoid arthritis clinical trials: introduction. *J Rheumatol.* 1993;20(3):528-530.
214. Morrison A, Polisena J, Husereau D, et al. The effect of English-language restriction on systematic review-based meta-analyses: a systematic review of empirical studies. *Int J Technol Assess Health Care.* 2012;28(2):138-144.
215. Weir CJ, Butcher I, Assi V, et al. Dealing with missing standard deviation and mean values in meta-analysis of continuous outcomes: a systematic review. *BMC Med Res Methodol.* 2018;18(1):25.
216. Higgins J, Green S. Handbook for systematic reviews of interventions version 5.1. 0 [updated March 2011]. The Cochrane Collaboration. 2011 Jul 25;5:252-8.
217. Schünemann H, Brożek J, Guyatt G, A O. Schunemann H. GRADE handbook for grading quality of evidence and strength of recommendation. Version 3.2. http://www. cc-ims. net/gradepro. 2008.Accessed on line Sept 2018
218. Alipour B, Homayouni-Rad A, Vaghef-Mehrabany E, et al. Effects of Lactobacillus casei supplementation on disease activity and inflammatory cytokines in rheumatoid arthritis patients: a randomized double-blind clinical trial. *Int J Rheum Dis.* 2014;17(5):519-527.
219. Brophy S, Burrows CL, Brooks C, Gravenor MB, Siebert S, Allen SJ. Internet-based randomised controlled trials for the evaluation of complementary and alternative medicines: probiotics in spondyloarthropathy. *BMC Musculoskelet Disord.* 2008;9:4.
220. Hatakka K, Martio J, Korpela M, et al. Effects of probiotic therapy on the activity and activation of mild rheumatoid arthritis--a pilot study. *Scand J Rheumatol.* 2003;32(4):211-215.
221. Jenks K, Stebbings S, Burton J, Schultz M, Herbison P, Highton J. Probiotic therapy for the treatment of spondyloarthritis: a randomized controlled trial. *J Rheumatol.* 2010;37(10):2118-2125.
222. Lee HJ, Waller RD, Stebbings S, et al. The effects of an orally administered probiotic on sulfasalazine metabolism in individuals with rheumatoid arthritis: a preliminary study. *Int J Rheum Dis.* 2010;13(1):48-54.
223. Mandel DR, Eichas K, Holmes J. Bacillus coagulans: a viable adjunct therapy for relieving symptoms of rheumatoid arthritis according to a randomized, controlled trial. *BMC Complement Altern Med.* 2010;10:1.

224. Pineda Mde L, Thompson SF, Summers K, de Leon F, Pope J, Reid G. A randomized, double-blinded, placebo-controlled pilot study of probiotics in active rheumatoid arthritis. *Med Sci Monit.* 2011;17(6):CR347-354.
225. Shukla A, Gaur P, Aggarwal A. Effect of probiotics on clinical and immune parameters in enthesitis-related arthritis category of juvenile idiopathic arthritis. *Clin Exp Immunol.* 2016;185(3):301-308.
226. Tomasello G, Margiotta G, Noto M, et al. Beneficial Effect of Probiotics Administration in Inflammatory Bowel Disease and Related Spondyloarthropathy: A Prospective Study. *Med SCi Tech.* 2015:100-103.
227. Vaghef-Mehrabany E, Alipour B, Homayouni-Rad A, Sharif SK, Asghari-Jafarabadi M, Zavvari S. Probiotic supplementation improves inflammatory status in patients with rheumatoid arthritis. *Nutrition.* 2014;30(4):430-435.
228. Zamani B, Golkar HR, Farshbaf S, et al. Clinical and metabolic response to probiotic supplementation in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. *Int J Rheum Dis.* 2016;19(9):869-879.
229. Zamani B, Farshbaf S, Golkar HR, Bahmani F, Asemi Z. Synbiotic supplementation and the effects on clinical and metabolic responses in patients with rheumatoid arthritis: a randomised, double-blind, placebo-controlled trial. *Br J Nutr.* 2017;117(8):1095-1102.
230. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Reprint--preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Phys Ther.* 2009;89(9):873-880.
231. Tufanaru C, Munn Z, Aromataris E, Campbell J, . HL. Chapter 3: Systematic reviews of effectiveness. In: Aromataris E, Z M, eds. *Joanna Briggs Institute Reviewer's Manual*. Adelaide: The Joanna Briggs Institute; 2017. https://wiki.joannabriggs.org/display/MANUAL/JBI+Reviewer%27s+Manual accessed online Feb 2017
232. Tointon A. The Pulse on Patient Recruitment : The issue of patient retention in clinical trials. In: centerwatch; 2016. https://www.centerwatch.com/news-online/2016/06/27/issue-patient-retention-clinical-trials/ Accessed online Oct 2018
233. Cella D, Yount S, Sorensen M, Chartash E, Sengupta N, Grober J. Validation of the Functional Assessment of Chronic Illness Therapy Fatigue Scale relative to other instrumentation in patients with rheumatoid arthritis. *J Rheumatol.* 2005;32(5):811-819.
234. Busse JW, Bartlett SJ, Dougados M, et al. Optimal Strategies for Reporting Pain in Clinical Trials and Systematic Reviews: Recommendations from an OMERACT 12 Workshop. *J Rheumatol.* 2015;42(10):1962-1970.
235. Olsen MF, Bjerre E, Hansen MD, et al. Pain relief that matters to patients: systematic review of empirical studies assessing the minimum clinically important difference in acute pain. . *BMC medicine.* 2017;15:35.
236. Cheng J, Pullenayegum E, Marshall JK, Iorio A, Thabane L. Impact of including or excluding both-armed zero-event studies on using standard meta-analysis methods for rare event outcome: a simulation study. *BMJ Open.* 2016;6(8):e010983.
237. McKean J, Naug H, Nikbakht E, Amiet B, Colson N. Probiotics and Subclinical Psychological Symptoms in Healthy Participants: A Systematic Review and Meta-Analysis. *J Altern Complement Med.* 2017;23(4):249-258.
238. Steenbergen L, Sellaro R, van Hemert S, Bosch JA, Colzato LS. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain Behav Immun.* 2015;48:258-264.

239. Messaoudi M, Lalonde R, Violle N, et al. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus R0052* and *Bifidobacterium longum R0175*) in rats and human subjects. *Br J Nutr.* 2011;105(5):755-764.
240. Wolfe F, Michaud K. Assessment of pain in rheumatoid arthritis: minimal clinically significant difference, predictors, and the effect of anti-tumor necrosis factor therapy. *J Rheumatol.* 2007;34(8):1674-1683.
241. Huang R, Wang K, Hu J. Effect of Probiotics on Depression: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients.* 2016;8(8).
242. Wallace CJK, Milev R. Erratum to: The effects of probiotics on depressive symptoms in humans: a systematic review. *Ann Gen Psychiatry.* 2017;16:18.
243. Bubnov RV, Babenko LP, Lazarenko LM, Mokrozub VV, Spivak MY. Specific properties of probiotic strains: relevance and benefits for the host. *EPMA J.* 2018;9(2):205-223.
244. Dinan TG, Cryan JF. The Microbiome-Gut-Brain Axis in Health and Disease. *Gastroenterol Clin North Am.* 2017;46(1):77-89.
245. Sudo N, Chida Y, Aiba Y, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol.* 2004;558(Pt 1):263-275.
246. Foster JA, Rinaman L, Cryan JF. Stress & the gut-brain axis: Regulation by the microbiome. *Neurobiol Stress.* 2017;7:124-136.
247. Rizzo A, Guggino G, Ferrante A, Ciccia F. Role of Subclinical Gut Inflammation in the Pathogenesis of Spondyloarthritis. *Front Med (Lausanne).* 2018;5:63.
248. Shen J, Zuo ZX, Mao AP. Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. *Inflamm Bowel Dis.* 2014;20(1):21-35.
249. Ganji-Arjenaki M, Rafieian-Kopaei M. Probiotics are a good choice in remission of inflammatory bowel diseases: A meta analysis and systematic review. *J Cell Physiol.* 2018;233(3):2091-2103.
250. Mielants H, Veys EM, Cuvelier C, de Vos M. Ileocolonoscopic findings in seronegative spondylarthropathies. *Br J Rheumatol.* 1988;27 Suppl 2:95-105.
251. Graham B, Green A, James M, Katz J, Swiontkowski M. Measuring patient satisfaction in orthopaedic surgery. *J Bone Joint Surg Am.* 2015;97(1):80-84.
252. Huang HY, Andrews E, Jones J, Skovron ML, Tilson H. Pitfalls in meta-analyses on adverse events reported from clinical trials. *Pharmacoepidemiol Drug Saf.* 2011;20(10):1014-1020.
253. Lee YJ, Ellenberg JH, Hirtz DG, Nelson KB. Analysis of clinical trials by treatment actually received: is it really an option? *Stat Med.* 1991;10(10):1595-1605.
254. Gueimonde M, Sanchez B, C GdLR-G, Margolles A. Antibiotic resistance in probiotic bacteria. *Front Microbiol.* 2013;4:202.
255. Hammad AM, Shimamoto T. Towards a compatible probiotic-antibiotic combination therapy: assessment of antimicrobial resistance in the Japanese probiotics. *J Appl Microbiol.* 2010;109(4):1349-1360.
256. Bafeta A, Koh M, Riveros C, Ravaud P. Harms Reporting in Randomized Controlled Trials of Interventions Aimed at Modifying Microbiota: A Systematic Review. *Ann Intern Med.* 2018;169(4):240-247.
257. Kopp MV, Goldstein M, Dietschek A, Sofke J, Heinzmann A, Urbanek R. Lactobacillus GG has in vitro effects on enhanced interleukin-10 and interferon-gamma release of mononuclear cells but no in vivo effects in supplemented mothers and their neonates. *Clin Exp Allergy.* 2008;38(4):602-610.

258. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol.* 2008;180(9):5771-5777.
259. McInnes IB, Buckley CD, Isaacs JD. Cytokines in rheumatoid arthritis - shaping the immunological landscape. *Nat Rev Rheumatol.* 2016;12(1):63-68.
260. Butto LF, Haller D. Functional relevance of microbiome signatures: The correlation era requires tools for consolidation. *J Allergy Clin Immunol.* 2017;139(4):1092-1098.
261. Rajilic-Stojanovic M, Heilig HG, Molenaar D, et al. Development and application of the human intestinal tract chip, a phylogenetic microarray: analysis of universally conserved phylotypes in the abundant microbiota of young and elderly adults. *Environ Microbiol.* 2009;11(7):1736-1751.
262. Claesson MJ, Cusack S, O'Sullivan O, et al. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc Natl Acad Sci U S A.* 2011;108 Suppl 1:4586-4591.
263. Biagi E, Nylund L, Candela M, et al. Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS One.* 2010;5(5):e10667.
264. Guigoz Y, Dore J, Schiffrin EJ. The inflammatory status of old age can be nurtured from the intestinal environment. *Curr Opin Clin Nutr Metab Care.* 2008;11(1):13-20.
265. Bartosch S, Woodmansey EJ, Paterson JC, McMurdo ME, Macfarlane GT. Microbiological effects of consuming a synbiotic containing Bifidobacterium bifidum, Bifidobacterium lactis, and oligofructose in elderly persons, determined by real-time polymerase chain reaction and counting of viable bacteria. *Clin Infect Dis.* 2005;40(1):28-37.
266. Tejesvi MV, Arvonen M, Kangas SM, et al. Faecal microbiome in new-onset juvenile idiopathic arthritis. *Eur J Clin Microbiol Infect Dis.* 2016;35(3):363-370.
267. Meinzer U, Faye A. Probiotic Treatment in Juvenile Idiopathic Arthritis ClinicalTrials.gov [Internet]. https://clinicaltrials.gov/show/NCT03092427,. Published 2017. Accessed.
268. van Nies JA, Krabben A, Schoones JW, Huizinga TW, Kloppenburg M, van der Helm-van Mil AH. What is the evidence for the presence of a therapeutic window of opportunity in rheumatoid arthritis? A systematic literature review. *Ann Rheum Dis.* 2014;73(5):861-870.
269. Efthimiou P, Kukar M, Mackenzie CR. Complementary and alternative medicine in rheumatoid arthritis: no longer the last resort! *HSS J.* 2010;6(1):108-111.
270. Carron P, Varkas G, Renson T, Colman R, Elewaut D, Van den Bosch F. High rate of drug-free remission after induction therapy with golimumab in early peripheral spondyloarthritis. *Arthritis Rheum.* 2018.
271. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol.* 2016;14(1):20-32.
272. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature.* 2006;444(7122):1022-1023.
273. Zmora N, Zeevi D, Korem T, Segal E, Elinav E. Taking it Personally: Personalized Utilization of the Human Microbiome in Health and Disease. *Cell Host Microbe.* 2016;19(1):12-20.
274. Borgeraas H, Johnson LK, Skattebu J, Hertel JK, Hjelmesaeth J. Effects of probiotics on body weight, body mass index, fat mass and fat percentage in subjects with overweight or obesity: a systematic review and meta-analysis of randomized controlled trials. *Obes Rev.* 2018;19(2):219-232.
275. Eder L, Thavaneswaran A, Chandran V, Cook RJ, Gladman DD. Obesity is associated with a lower probability of achieving sustained minimal disease activity state among patients with psoriatic arthritis. *Ann Rheum Dis.* 2015;74(5):813-817.

276. Nikiphorou E, Norton S, Carpenter L, et al. Secular Changes in Clinical Features at Presentation of Rheumatoid Arthritis: Increase in Comorbidity But Improved Inflammatory States. *Arthritis Care Res (Hoboken).* 2017;69(1):21-27.
277. Davis SC, Yadav JS, Barrow SD, Robertson BK. Gut microbiome diversity influenced more by the Westernized dietary regime than the body mass index as assessed using effect size statistic. *Microbiologyopen.* 2017;6(4).
278. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science.* 2011;334(6052):105-108.
279. Liang X, Bittinger K, Li X, Abernethy DR, Bushman FD, FitzGerald GA. Bidirectional interactions between indomethacin and the murine intestinal microbiota. *Elife.* 2015;4:e08973.
280. Montalto M, Gallo A, Curigliano V, et al. Clinical trial: the effects of a probiotic mixture on non-steroidal anti-inflammatory drug enteropathy - a randomized, double-blind, cross-over, placebo-controlled study. *Aliment Pharmacol Ther.* 2010;32(2):209-214.
281. Otani K, Tanigawa T, Watanabe T, et al. Microbiota Plays a Key Role in Non-Steroidal Anti-Inflammatory Drug-Induced Small Intestinal Damage. *Digestion.* 2017;95(1):22-28.
282. Sayers E, MacGregor A, Carding SR. Drug-microbiota interactions and treatment response: Relevance to rheumatoid arthritis. *AIMS Microbiol.* 2018;4(4):642-654.
283. Mikkelsen KH, Frost M, Bahl MI, et al. Effect of Antibiotics on Gut Microbiota, Gut Hormones and Glucose Metabolism. *PLoS One.* 2015;10(11):e0142352.
284. Mikov M, Lee HJ, JP. F. The influence of probiotic treatment on sulfasalazine metabolism in rat gut contents. *Asian J Pharmacokinet Pharmacodynam.* 2006;6:337-342.
285. Watanabe T, Nishio H, Tanigawa T, et al. Probiotic Lactobacillus casei strain Shirota prevents indomethacin-induced small intestinal injury: involvement of lactic acid. *Am J Physiol Gastrointest Liver Physiol.* 2009;297(3):G506-513.
286. Madsen K, Cornish A, Soper P, et al. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology.* 2001;121(3):580-591.
287. Zmora N, Zilberman-Schapira G, Suez J, et al. Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. *Cell.* 2018;174(6):1388-1405 e1321.
288. Bertazzoni E, Donelli G, Midtvedt T, Nicoli J, Sanz Y. Probiotics and clinical effects: is the number what counts? *J Chemotherapy.* 2013;25(4):193-212.
289. Taverniti V, Koirala R, Dalla Via A, et al. Effect of Cell Concentration on the Persistence in the Human Intestine of Four Probiotic Strains Administered through a Multispecies Formulation. *Nutrients.* 2019;11(2).
290. Chen LL, Zou YY, Lu FG, Li FJ, Lian GH. Efficacy profiles for different concentrations of Lactobacillus acidophilus in experimental colitis. *World J Gastroenterol.* 2013;19(32):5347-5356.
291. Fenster K, Freeburg B, Hollard C, Wong C, Ronhave Laursen R, Ouwehand AC. The Production and Delivery of Probiotics: A Review of a Practical Approach. *Microorganisms.* 2019;7(3).
292. Goldstein EJ, Citron DM, Claros MC, Tyrrell KL. Bacterial counts from five over-the-counter probiotics: are you getting what you paid for? *Anaerobe.* 2014;25:1-4.
293. Drago L, Rodighiero V, Celeste T, Rovetto L, E. DV. Microbiological evaluation of commercial probiotic products available in the USA in 2009. *J Chemotherapy.* 2010;22:373-377.

294. Kolacek S, Hojsak I, Berni Canani R, et al. Commercial Probiotic Products: A Call for Improved Quality Control. A Position Paper by the ESPGHAN Working Group for Probiotics and Prebiotics. *J Pediatr Gastroenterol Nutr.* 2017;65(1):117-124.
295. Lewis ZT, Shani G, Masarweh CF, et al. Validating bifidobacterial species and subspecies identity in commercial probiotic products. *Pediatr Res.* 2016;79(3):445-452.
296. Mills DA. Probiotic nomenclature matters redux: confusion on Bifidobacterium longum subsp. infantis taxonomy persists. *Curr Med Res Opin.* 2017;33(11):2097.
297. van der Aa Kühle A, Jespersen L. The taxonomic position of Saccharomyces boulardii as evaluated by sequence analysis of the D1/D2 domain of 26S rDNA, the ITS1-5.8 S rDNA-ITS2 region and the mitochondrial cytochrome-c oxidase II gene. *Syst Appl Microbiol.* 2003;26(4):564-571.
298. Di Pierro F, Colombo M, Zanvit A, Risso P, Rottoli AS. Use of Streptococcus salivarius K12 in the prevention of streptococcal and viral pharyngotonsillitis in children. *Drug Healthc Patient Saf.* 2014;6:15-20.
299. Jamali Z, Aminabadi NA, Samiei M, Sighari Deljavan A, Shokravi M, Shirazi S. Impact of Chlorhexidine Pretreatment Followed by Probiotic Streptococcus salivarius Strain K12 on Halitosis in Children: A Randomised Controlled Clinical Trial. *Oral Health Prev Dent.* 2016;14(4):305-313.
300. Miranda LA, Fischer RG, Sztajnbok FR, Figueredo CM, Gustafsson A. Periodontal conditions in patients with juvenile idiopathic arthritis. J Clin Periodontol. 2003;30(11):969-974.
301. Silvestre-Rangil J, Bagan L, Silvestre FJ, Bagan JV. Oral manifestations of rheumatoid arthritis. A cross-sectional study of 73 patients. *Clin Oral Investig.* 2016;20(9):2575-2580.
302. Wells JM. Immunomodulatory mechanisms of lactobacilli. *Microb Cell Fact.* 2011;10 Suppl 1:S17.
303. Vlasova AN, Kandasamy S, Chattha KS, Rajashekara G, Saif LJ. Comparison of probiotic lactobacilli and bifidobacteria effects, immune responses and rotavirus vaccines and infection in different host species. *Vet Immunol Immunopathol.* 2016;172:72-84.
304. Coppen A, Bolander-Gouaille C. Treatment of depression: time to consider folic acid and vitamin B12. *J Psychopharmacol.* 2005;19(1):59-65.
305. LeBlanc JG, Laino JE, del Valle MJ, et al. B-group vitamin production by lactic acid bacteria--current knowledge and potential applications. *J Appl Microbiol.* 2011;111(6):1297-1309.
306. Bell ML, Kenward MG, Fairclough DL, Horton NJ. Differential dropout and bias in randomised controlled trials: when it matters and when it may not. *BMJ.* 2013;346:e8668.
307. Schmid CH. Discussion of "quantifying publication bias in meta-analysis" by Lin et al. *Biometrics.* 2018;74(3):797-799.
308. Saa C, Bunout D, Hirsch S. Industry funding effect on positive results of probiotic use in the management of acute diarrhea: a systematized review. *Eur J Gastroenterol Hepatol.* 2019;31(3):289-302.
309. Maddaloni M, Kochetkova I, Hoffman C, Pascual D. Delivery of IL-35 by Lactococcus lactis Ameliorates Collagen-Induced Arthritis in Mice. *Front Immunol.* 2018;9:2691.
310. Andrews NA, Latremoliere A, Basbaum AI, et al. Ensuring transparency and minimization of methodologic bias in preclinical pain research: PPRECISE considerations. *Pain.* 2016;157(4):901-909.

Appendices

Appendix Ia JBI Critical appraisal tool randomised control trials

JBI Critical Appraisal Checklist for Randomized Controlled Trials

Reviewer_____Date_____

Author _____Year_____Record Number _____

		Yes	No	Unclear	NA
1.	Was true randomization used for assignment of participants to treatment groups?	☐	☐	☐	☐
2.	Was allocation to treatment groups concealed?	☐	☐	☐	☐
3.	Were treatment groups similar at the baseline?	☐	☐	☐	☐
4.	Were participants blind to treatment assignment?	☐	☐	☐	☐
5.	Were those delivering treatment blind to treatment assignment?	☐	☐	☐	☐
6.	Were outcomes assessors blind to treatment assignment?	☐	☐	☐	☐
7.	Were treatment groups treated identically other than the intervention of interest?	☐	☐	☐	☐
8.	Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	☐	☐	☐	☐
9.	Were participants analyzed in the groups to which they were randomized?	☐	☐	☐	☐
10.	Were outcomes measured in the same way for treatment groups?	☐	☐	☐	☐
11.	Were outcomes measured in a reliable way?	☐	☐	☐	☐
12.	Was appropriate statistical analysis used?	☐	☐	☐	☐
13.	Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

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Appendix Ib Modified version of JBI Critical appraisal tool for randomised control trials

Study:

1. **Was true randomisation used for assignment of participants to treatment groups?**

Yes	Method by which randomisation to intervention or control group described eg random allocation using number generator, stratified block randomisation scheme
No	Method other than randomisation used to allocate patients to groups (quasi randomisation/stratification)
Unclear	Terms like 'random' and 'randomisation' used but method not described

Comment/detail: _____

2. **Was allocation to treatment groups concealed?**

Yes	The researcher responsible for allocating participants to compared groups is unaware of the allocation order. An appropriate allocation concealment method was used, such as central randomisation; sequentially numbered opaque sealed envelopes used?
No	Person responsible for allocation to groups able to determine which group the participant was being allocated.
Unclear	Unable to determine how allocation to treatment groups occurred.

Comment: _____

3. **Were treatment groups similar at the baseline?**

Yes	Recorded minimum base line data including: - Age - Gender ratio - Duration of disease (time since diagnosis) - Severity of disease (i.e. mild, moderate or severe) - Baseline measurements for individual intended measurable outcomes Additional information can include - Weight - diet - concurrent medication use (i.e. oral steroids , intra artic steroids, NSAIDS or DMARDS) - Genetic or serum disease markers (HLAB27, Rheumatoid factor, ACCP ore presence of enthesitis)
No	Baseline data between groups is clearly not comparable (statistical differences between groups at baseline that may affect the outcome of results eg differences in sex, age, SES on effectiveness of intervention/uptake)
Unclear	Inadequately described. No or minimal reporting of baseline data i.e. only age, sex. No mention of statistical difference between groups where differences in baseline data are apparent.

Comment: _____

4. **Were participants blind to treatment assignment?**

Yes	Participants unaware that they have been allocated to either intervention or control group and methods for ensuring participant blinding to treatment assignment indicated. *(provision of placebo pills in same format is necessary to ensure blinding)
No	Participants aware of which group they have been allocated
Unclear	Inadequately described

Comment: _____

5. **Were those delivering the treatment blind to treatment assignment?**

Yes	Doctors/nurses/health workers implementing the intervention are unaware if they are providing intervention to control or intervention/treatment group

No	Doctors/nurses/health workers implementing the intervention are aware they are providing the intervention to the treatment group Provide explanation for lack of blinding
Unclear	Inadequately described

Comment: _____

6. Were outcomes assessors blind to treatment assignment?

Yes	Data collectors were blinded for outcomes assessment (eg conducting interview)
No	Data collectors were aware of the group in which the participant belonged
Unclear	Inadequately described

Comment: _____

7. Were treatment groups treated identically other than the intervention of interest?

Yes	Participants in both the intervention and control groups were treated identically for all other aspects other than intervention of interest , eg access to ongoing medication as required, exercise and usual diet. Study includes active control group. Any intervention provided to control group described in detail, e.g. control group received daily placebo tablet if the intervention received daily probiotic tablet
No	Wait listed control groups where control intervention not provided, or intervention provided clearly not matched for attention, giving control participants an indication that they may be in the control group. Participants receiving concurrent intervention outside intervention of interest that may impact on results, eg allowed to take other supplements, to use antibiotics or start medication such as Biological class DMARDS
Unclear	Control intervention inadequately described

Comment: _____

8. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analysed?

Yes	Complete follow up Withdrawn participants/losses to follow up reported and reasons for the withdrawal described. All participants included in final calculations including withdrawn participants, regardless of whether their final outcomes were measured.
No	No explanation of withdrawn participants/losses to follow up, or the significance of these withdrawals. Withdrawn participants not analysed in the groups to which they were allocated
Unclear	Withdrawn participants inadequately described Numbers of included/withdrawn participants do not match result figures, inadequately described

Comment: _____

9. Were participants analysed in the groups to which they were randomised?

Yes	Withdrawn participants analysed in the groups to which they were originally allocated (Intention to treat analysis, ITT)
No	Missing participant data not reported or accounted for
Unclear	Inadequately described

Comment: _____

10. Were outcomes measured in the same way for treatment groups?

Yes	Outcome data was measured and collected consistently in all groups
No	Outcome data was measured and collected differently for each group
Unclear	Inadequately described

Comment: _____

11. Were outcomes measured in a reliable way?

Yes	Outcomes (Pain, serum markers of inflammation such as CRP or ESR, clinical outcomes such as DAS28 or immunological markers) measured using standardised methods - Authors identify tool adequately- for example Composite scores may be created with ESR or CRP values – have they identified which were used? - Authors state the reliability and or validity of the measures used (incl appropriately trained clinical data collectors) or piloted within the trial. - Demonstrates/indicates test-retest reliability (for example Global scores undertaken by the clinician alone have very poor inter-rater reliability) - Are clear about when subjective measures such as pain (VAS) are being assessed (as a point measurement at a single clinical visit? Or by recall for a past period)
No	Self-reported/subjective outcomes, reliant on participant recall - VAS over past days/week or months - Patient self-report of compliance with taking the probiotic/placebo tablets No reporting on the reliability and or validity of the methods used No indication of outcomes tools being employed by individuals trained in their use.
Unclear	Inadequately described

Comment: _____

12. Was appropriate statistical analysis used?

Yes	Appropriate statistical methods used, described and reported For example, Paired or nonnon-paired data tests Mean difference reported for comparison between intervention and active control
No	Statistical methods inappropriate
Unclear	Inadequately described

Comment: _____

13. Was the trial design appropriate and any deviations from the standard RCT design (individual randomisation, parallel groups) accounted for in the conduct and analysis of the trial?

Yes	Described study methods in detail and any deviation from standard RCT design accounted for with explanation
No	Inappropriate study design/no explanation of deviation from standard RCT
Unclear	Inadequately described, lack of detail

Comment: _____

Appendix IIa JBI Critical appraisal tool- Quasi-experimental studies (non-randomised)

JBI Critical Appraisal Checklist for Quasi-Experimental Studies (non-randomized experimental studies)

Reviewer_____Date_____

Author _____Year_____Record Number _____

		Yes	No	Unclear	Not applicable
1.	Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)?	☐	☐	☐	☐
2.	Were the participants included in any comparisons similar?	☐	☐	☐	☐
3.	Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?	☐	☐	☐	☐
4.	Was there a control group?	☐	☐	☐	☐
5.	Were there multiple measurements of the outcome both pre and post the intervention/exposure?	☐	☐	☐	☐
6.	Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	☐	☐	☐	☐
7.	Were the outcomes of participants included in any comparisons measured in the same way?	☐	☐	☐	☐
8.	Were outcomes measured in a reliable way?	☐	☐	☐	☐
9.	Was appropriate statistical analysis used?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

_____

_____

_____

Appendix IIb Modified version of JBI Critical appraisal tool quasi experimental (non-randomised controlled) trials

Study:

14. **Is it clear in the study what is cause and what is effect?**

Yes	It is clear that the 'cause' – ie provision of probiotics was manipulated before the occurrence of the 'effect'? (taken to be the patient reported outcomes)
No	It is NOT clear that the 'cause' – ie provision of probiotics was manipulated before the occurrence of the 'effect'? (taken to be the patient reported outcomes)
Unclear	There is lack of clarity about the timelines for intervention and measuring outcomes.

Comment/detail:_____

15. **Were the participants included in the comparison similar?**

Yes	Recorded minimum base line data shows similarity including: - Age - Gender ratio - Duration of disease (time since diagnosis) - Severity of disease (i.e. mild, moderate or severe) - Baseline measurements for individual intended measurable outcomes Additional information can include - Weight - diet - concurrent medication use (i.e. steroid (oral or injected) NSAIDS or DMARDS) Genetic or serum disease markers (HLAB27, Rheumatoid factor, etc
No	Baseline data between groups is clearly not comparable (statistical differences between groups at baseline that may affect the outcome of results eg differences in sex, age, SES on effectiveness of intervention/uptake)
Unclear	Inadequately described. No or minimal reporting of baseline data i.e. only age, sex. No mention of statistical differences in baseline data between groups.

Comment:_____

16. **Were the participants included in any comparisons receiving the similar treatment or care to other than the exposure or intervention of interest ?**

Yes	Participants in both the intervention and control groups were treated identically for all other aspects other than intervention of interest , eg access to ongoing medication as required, exercise and usual diet. Study includes active control group. Any intervention provided to control group described in detail, e.g. control group received daily placebo tablet if the intervention received daily probiotic tablet
No	Wait listed control groups where control intervention not provided, or intervention provided clearly not matched for attention, giving control participants an indication that they may be in the control group. Participants receiving concurrent intervention outside intervention of interest that may impact on results, eg allowed to take other supplements, to use antibiotics or start medication such as Biological class DMARDS
Unclear	Control intervention inadequately described

Comment: _____

17. **Was there a control group?**

Yes	An independent control group was used
No	NO an independent control was not used
Unclear	Note that The control group should be an independent, separate control group, not the pre-test group in a single group pre-test post-test design.

Comment_____

18. Were there multiple measurements of the outcome both pre and post intervention?

Yes	multiple measurements of the outcome both pre and post intervention
No	No measures pre or post were lacking
Unclear	Inadequately described

Comment: _____

19. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analysed?

Yes	Complete follow up Withdrawn participants/losses to follow up reported and reasons for the withdrawal described. All participants included in final calculations including withdrawn participants, regardless of whether their final outcomes were measured.
No	No explanation of withdrawn participants/losses to follow up, or the significance of these withdrawals. Withdrawn participants not analysed in the groups to which they were allocated
Unclear	Withdrawn participants inadequately described. Numbers of included/withdrawn participants do not match result figures, inadequately described

Comment: _____

20. Were outcomes of participants included in any comparison measured in the same way?

Yes	Outcome data was measured and collected consistently in all groups
No	Outcome data was measured and collected differently for each group
Unclear	Inadequately described

Comment: _____

21. Were outcomes measured in a reliable way?

Yes	Outcomes (Pain, serum markers of inflammation such as CRP or ESR, clinical outcomes such as DAS28 or immunological markers) measured using standardised methods - Authors identify tool adequately- for example Composite scores may be created with ESR or CRP values – have they identified which were used? - Authors state the reliability and or validity of the measures used (incl appropriately trained clinical data collectors) or piloted within the trial. - Demonstrates/indicates test-retest reliability (for example Global scores undertaken by the clinician alone have very poor inter-rater reliability) - Are clear about when subjective measures such as pain (VAS) are being assessed (as a point measurement at a single clinical visit? Or by recall for a past period)
No	Self-reported/subjective outcomes, reliant on participant recall - VAS over past days/week or months - Patient self-report of compliance with taking the probiotic/placebo tablets No reporting on the reliability and or validity of the methods used No indication of outcomes tools being employed by individuals trained in their use.
Unclear	Inadequately described

Comment: _____

22. Was appropriate statistical analysis used?

Yes	Appropriate statistical methods used, described and reported For example, Paired or non-paired data tests Mean difference reported for comparison between intervention and active control
No	Statistical methods inappropriate
Unclear	Inadequately described

Comment_____

Appendix III Data Extraction template

Drop out

Study	Total N	Start N		finish N		Drop out%		
		probiotic	control	Probiotic	control	probiotic	control	Total
Alipour et al	*60*	*30*	*30*	*22*	*24*	*26*	*20*	*23*

Population demographics

Study	AGE -mean Yrs. (SD)		Disease duration mean YRS (SD)		Gender ratio M:F		% HLAB27		other
	probiotic	control	Probiotic	control	probiotic	control	probiotic	control	
Alipour et al	*41.4 (SD 12.65)*	*44.29 (SD 9.77)*	*6.06 (SD 1.81)*	*5.88 (SD1.7)*	*0:30*	*0:30*	*N/A*	*N/A*	

Probiotic intervention

Probiotic	Avg CFU over all studies 10^8	N (receiving) genera	N (receiving) species	MIN CFU	MAX CFU	*Alipour B, et al*	
Saccharomyces boulardii	N/A	31	31				
Bifidobacterium lactis	8	312	44				
Bifidobacterium breve	N/A		23				
Bifidobacterium Longum	N/A		23				
Bifidobacterium infantis	12.5		94				
Bifidobacterium bifidum	16.25		128	$12.5x\ 10^8$	20×10^8		
Lactobacillus acidophilus	N/A	515	155	4×10^8	$2x\ 10^9$		
Lactobacillus salivarius	N/A		102				
Lactobacillus rhamnosus	210		27	10x108	$400x10^8$		
Lactobacillus plantarum	14		23				
Lactobacillus paracasei	6.25		94				
Lactobacillus delbrueckii	N/A		23				
Lactobacillus caseii	N/A		77	1×10^8	20×10^8	*$1x\ 10^8$*	
Lactobacillus reuterii	12.5		14				
Streptococcus thermophilus	N/A	97	23				
Streptococcus salivarius	13.6		74	1×10^8	8×10^8		
Enterococcus faecium	20	31	31				
Bacillus coagulans	150	22	22				
Delivery						*Once daily*	
Total per study in CFU						*$1x\ 10^8$*	
Total per study CFU 10^8						*$1x\ 10^8$*	

*N/A CFU not supplied for all studies so all study average not calculable

** Whilst a variety of CFU supplied all CFU stated in 10^8 to make comparable and allow average calculation

Disease duration

Study	AGE -mean Yrs. (SD)		Disease duration mean YRS (SD)		Gender ratio M:F		HLAB27 as percentage	
	probiotic	control	Probiotic	control	probiotic	control	probiotic	control
Alipour et al	*41.4 (SD 12.65)*	*44.29 (SD 9.77)*	*6.06 (SD 1.81)*	*5.88 (SD1.7)*	*0:30*	*0:30*	*Not measured*	*Not measured*

Exclusions

Study	Lifestyle					Conditions					
	Probiotic use	Lactose intol.	diet	smoker	obese	Pregnant lactating	Thyroid	IBD	Kidney	Liver	DM
Alipour et al	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*

Study	Medications					
	antibiotics	NSAIDS	Oral steroids	DMARDS	bDMARDS	Other (i.e. HFT)
Alipour et al	*X*	*X*	*Not stated*	*Not stated*	*X*	*X*

OUTCOME – Life impact – Bowel symptom

Study	Tool	Symptom	Probiotic Mean (SD)		Control Mean (SD)		Stats	
			baseline	end	baseline	end	Effect size	range
Brophy et al	*DISQ*	*diarrhoea*	*1.8 (SD1.6)*	*1.4(SD2.3)*	*1.9(SD2.7)*	*1(SD1.7)*	*0.24*	*(-0.36, 0.83)*

*Repeated for Pain, Blood in stools

OUTCOME – Life impact – PAIN

Study	Tool	Pain mean(SD) Probiotic		Pain Mean (SD) Control		Stats	
		baseline	end	baseline	end	Effect size	Range
Jenks et al	*VAS*	*29 (SD23)*	*27 (SD25)*	*30 (SD26)*	*26 (SD22)*	*0.0424*	*(-0.45, 0.54)*

OUTCOME – Life impact – FATIGUE

Study	Tool	Outcome Probiotic		Outcome Control		Stats	
		baseline	End	baseline	End	Effect size	Range
Jenks et al	*MAFS*	*24.3 (SD11.7)*	*21.9 (SD10.2)*	*25.8 (SD11.4)*	*23.9 (SD11.1)*	*-0.19*	*(-0.68,0.31)*

OUTCOME – Life impact – quality of life

Study	Tool	Score probiotic mean (SD)		Score Control mean (SD)		Stats	
		baseline	end	baseline	end	Effect size	Range
Brophy et al	*BAS-G*	*3.2 (SD 0.2)*	*2.9 (SD2.3)*	*4.1(SD2.5)*	*3.7(SD0)*	*0.16*	*(-0.16,0.93)*

OUTCOME – Adverse effects

Study	Sample size		Serious adverse effects		Minor adverse effects		Relative risk (Any adverse event)
	probiotic	control	probiotic	control	Probiotic	control	
Brophy et al	*76*	*71*	*0*	*0*	*6*	*5*	*1.12*

OUTCOME – Disease manifestations – systemic inflammation

Study	Marker	Outcome Probiotic		Outcome Control		Stats	
		baseline	End	baseline	End	Effect size	range
Jenks et al	*CRP*	*6.6(SD6.7)*	*6.7 (SD6.3)*	*10 (SD11.3)*	*11.3 (SD11.2)*	*0.504*	*(-1.01,-0.01)*

Appendix IV Excluded studies

		EXCLUSION
1	Australian Rheumatology Association in Conjunction with Rheumatology Health Professionals Association 51st Annual Scientific Meeting. Internal Medicine Journal. 2010;40.	Discussion- no supply probiotic
2	Can gut bacteria improve your health? Harvard Men's Health Watch. 2016;21(4):1-7.	Discussion- no supply probiotic
3	Abdollahi-Roodsaz S, Abramson SB, Scher JU. The metabolic role of the gut microbiota in health and rheumatic disease: mechanisms and interventions. Nat Rev Rheumatol. 2016;12(8):446-55.	Discussion- no supply probiotic
4	Alavi A, Goodfellow L, Fraser O, Tarelli E, Bland M, Axford J. A double-blind, randomized, placebo-controlled study to explore the efficacy of a dietary plant-derived polysaccharide supplement in patients with rheumatoid arthritis. Rheumatology (Oxford). 2011;50(6):1111-9.	prebiotic only
5	Alberda C, Gramlich L, Meddings J, Field C, McCargar L, Kutsogiannis D, et al. Effects of probiotic therapy in critically ill patients: A randomized, double-blind, placebo-controlled trial. American Journal of Clinical Nutrition. 2007;85(3):816-23.	not Inflammatory Arthritis
6	Altomare R, Damiano G, Gioviale MC, Palumbo VD, Maione C, Spinelli G, et al. The intestinal ecosystem and probiotics. Progress in Nutrition. 2016;18(1):8-15.	Discussion- no supply probiotic
7	Aqaeinezhad Rudbane SM, Rahmdel S, Abdollahzadeh SM, Zare M, Bazrafshan A, Mazloomi SM. The efficacy of probiotic supplementation in rheumatoid arthritis: a meta-analysis of randomized, controlled trials. Inflammopharmacology. 2018;26(1):67-76.	Discussion- no supply probiotic
8	Bansal T, Garg S. Probiotics: From functional foods to pharmaceutical products. Current Pharmaceutical Biotechnology. 2008;9(4):267-87.	Discussion- no supply probiotic
9	Bedaiwi MK, Inman RD. Microbiome and probiotics: link to arthritis. Curr Opin Rheumatol. 2014;26(4):410-5.	Discussion- no supply probiotic
10	Bennett DM, Shekhel T, Radelet M, Miller MD. Isolated Lactobacillus chronic prosthetic knee infection. Orthopedics. 2014;37(1):e83-6.	not Inflammatory Arthritis
11	Berntson L. Anti-inflammatory effect by exclusive enteral nutrition (EEN) in a patient with juvenile idiopathic arthritis (JIA): brief report. Clin Rheumatol. 2014;33(8):1173-5.	Not probiotic
12	Berntson L, Agback P, Dicksved J. Changes in fecal microbiota and metabolomics in a child with juvenile idiopathic arthritis (JIA) responding to two treatment periods with exclusive enteral nutrition (EEN). Clin Rheumatol. 2016;35(6):1501-6.	Not probiotic
13	Berntson L, Hedlund-Treutiger I, Alving K. Anti-inflammatory effect of exclusive enteral nutrition in patients with juvenile idiopathic arthritis. Clin Exp Rheumatol. 2016;34(5):941-5.	Not probiotic
14	Bhakta NR. Pitfalls of probiotics. Science Translational Medicine. 2016;8(368).	Murine
15	Bhardwaj SB. Gut flora and its modification as a therapy. Reviews in Medical Microbiology. 2013;24(3):52-4.	Not probiotic
16	Bravo-Blas A, Wessel H, Milling S. Microbiota and arthritis: correlations or cause? Curr Opin Rheumatol. 2016;28(2):161-7.	Discussion- no supply probiotic
17	Bush LM, De Almeida KNF, Martin G, Perez MT. Probiotic-associated bifidobacterium septic prosthetic joint arthritis. Infectious Diseases in Clinical Practice. 2014;22(4):e39-e41.	not Inflammatory Arthritis
18	Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, et al. Nutrition and inflammatory processes. Proceedings of the Nutrition Society. 2008;67(OCE):E9.	Discussion- no supply probiotic
19	Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, et al. Inflammatory disease processes and interactions with nutrition. British Journal of Nutrition. 2009;101(SUPPL.S1):S1-S45.	Not probiotic

20	Cammarota G, Ianiro G, Cianci R, Bibbò S, Gasbarrini A, Currò D. The involvement of gut microbiota in inflammatory bowel disease pathogenesis: Potential for therapy. Pharmacology and Therapeutics. 2015;149:191-212.	not Inflammatory Arthritis
21	Ceccarelli G, Vullo V, d'Ettorre G. Single-strain versus multistrain probiotic supplementation treatment strategy for rheumatoid arthritis: comment on the article by Marietta et al. Arthritis and Rheumatology. 2018;70(2):320-1.	Letter re murine study
22	Cénit MC, Matzaraki V, Tigchelaar EF, Zhernakova A. Rapidly expanding knowledge on the role of the gut microbiome in health and disease. Biochimica et Biophysica Acta - Molecular Basis of Disease. 2014;1842(10):1981-92.	Discussion- no supply probiotic
23	Cerrato PL. Can "healthy" bacteria ward off disease? RN. 2000;63(4):71-4.	Discussion- no supply probiotic
24	Chatfield SM, Dharmage SC, Boers A, Martin BJ, Buchanan RR, Maksymowych WP, et al. Complementary and alternative medicines in ankylosing spondylitis: a cross-sectional study. Clin Rheumatol. 2009;28(2):213-7.	Not probiotic
25	Chiavaroli C, Moore A. An hypothesis to link the opposing immunological effects induced by the bacterial lysate OM-89 in urinary tract infection and rheumatoid arthritis. BioDrugs. 2006;20(3):141-9.	Discussion- no supply probiotic
26	Chow J, Mazmanian SK. Getting the Bugs out of the Immune System: Do Bacterial Microbiota "Fix" Intestinal T Cell Responses? Cell Host and Microbe. 2009;5(1):8-12.	Not probiotic
27	Clancy R. Immunobiotics and the probiotic evolution. FEMS Immunology and Medical Microbiology. 2003;38(1):9-12.	Discussion- no supply probiotic
28	Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease. BMJ (Online). 2018;360.	Discussion- no supply probiotic
29	Collado MC, Gueimonde M, Pérez-Martínez G. Current and future applications of probiotics. Current Nutrition and Food Science. 2011;7(3):170-80.	Discussion- no supply probiotic
30	Cooksey R, Brophy S, Gravenor MB, Brooks CJ, Burrows CL, Siebert S. Frequency and characteristics of disease flares in ankylosing spondylitis. Rheumatology. 2010 Feb 1;49(5):929-32.	Discussion- no supply probiotic
31	Costello ME, Robinson PC, Benham H, Brown MA. The intestinal microbiome in human disease and how it relates to arthritis and spondyloarthritis. Best Pract Res Clin Rheumatol. 2015;29(2):202-12.	Discussion- no supply probiotic
32	de Oliveira GLV, Leite AZ, Higuchi BS, Gonzaga MI, Mariano VS. Intestinal dysbiosis and probiotic applications in autoimmune diseases. Immunology. 2017;152(1):1-12.	Discussion- no supply probiotic
33	de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol. 2008;111:1-66.	Discussion- no supply probiotic
34	Decker C. Lactobacillus casei Supplementation Improves Inflammatory Markers and Disease Activity Scores in Rheumatoid Arthritis. Integrative Medicine Alert. 2015;18(5):56-8.	duplication of article by Vaghref-Mehraby
35	Dejoras EMM, Remalante PPM, Santiago ATM. Probiotic supplementation and its effect on disease activity in rheumatoid arthritis: A systematic review and meta-analysis. International Journal of Rheumatic Diseases. 2017;20:92.	Discussion- no supply probiotic
36	Di Cerbo A, Palmieri B, Aponte M, Morales-Medina JC, Iannitti T. Mechanisms and therapeutic effectiveness of lactobacilli. Journal of Clinical Pathology. 2016;69(3):187-203.	Discussion- no supply probiotic
37	Diamanti AP, Manuela Rosado M, Lagana B, D'Amelio R. Microbiota and chronic inflammatory arthritis: an interwoven link. J Transl Med. 2016;14(1):233.	Discussion- no supply probiotic
38	Diamanti AP, Manuela Rosado M, Laganà B, D'Amelio R. Microbiota and chronic inflammatory arthritis: An interwoven link. Journal of Translational Medicine. 2016;14(1).	Discussion- no supply probiotic

39	Dieleman LA. Additive effects of antibiotics, probiotics and prebiotics. EOS Rivista di Immunologia ed Immunofarmacologia. 2004;24(1-2):89-90.	Discussion- no supply probiotic
40	Doron S, Gorbach SL. Probiotics: their role in the treatment and prevention of disease. Expert Rev Anti Infect Ther. 2006;4(2):261-75.	not Inflammatory Arthritis
41	Doron S, Snydman DR, Gorbach SL. Lactobacillus GG: Bacteriology and clinical applications. Gastroenterology Clinics of North America. 2005;34(3):483-98.	Discussion- no supply probiotic
42	Dwivedi M, Kumar P, Laddha NC, Kemp EH. Induction of regulatory T cells: A role for probiotics and prebiotics to suppress autoimmunity. Autoimmunity Reviews. 2016;15(4):379-92.	not Inflammatory Arthritis
43	Ernst E. Complementary treatments in rheumatic diseases. Rheum Dis Clin North Am. 2008;34(2):455-67.	Not probiotic
44	Ezendam J, van Loveren H. Probiotics: immunomodulation and evaluation of safety and efficacy. Nutrition Reviews. 2006;64(1):1-14.	Discussion- no supply probiotic
45	Fernández-Llanio Comella N, Fernández Matilla M, Castellano Cuesta JA. Have complementary therapies demonstrated effectiveness in rheumatoid arthritis? Reumatologia Clinica. 2016;12(3):151-7.	Discussion- no supply probiotic
46	Fijan S. Microorganisms with claimed probiotic properties: An overview of recent literature. International Journal of Environmental Research and Public Health. 2014;11(5):4745-67.	Discussion- no supply probiotic
47	Fung I, Garrett JPD, Shahane A, Kwan M. Do bugs control our fate? the influence of the microbiome on autoimmunity. Current Allergy and Asthma Reports. 2012;12(6):511-9.	Discussion- no supply probiotic
48	Gaston JSH. Recent advances in understanding spondyloarthritis. F1000Research. 2017;6.	Discussion- no supply probiotic
49	Gill H, Prasad J. Probiotics, immunomodulation, and health benefits. Adv Exp Med Biol. 2008;606:423-54.	Discussion- no supply probiotic
50	Goldin BR, Gorbach SL. Clinical indications for probiotics: an overview. Clin Infect Dis. 2008;46 Suppl 2:S96-100; discussion S44-51.	not Inflammatory Arthritis
51	Gorbach SL. Probiotics in the Third Millennium. Digestive and Liver Disease. 2002;34(SUPPL. 2):S2-S7.	Discussion- no supply probiotic
52	Groeger D, O'Mahony L, Murphy EF, et al. Bifidobacterium infantis 35624 modulates host inflammatory processes beyond the gut. *Gut Microbes* 2013;4:325-39. doi:10.4161/gmic.25487 pmid:23842110.	not Inflammatory Arthritis
53	Grover HS, Luthra S. Probiotics - the nano soldiers of oral health. Journal, Indian Academy of Clinical Medicine. 2012;13(1):48-54.	Discussion- no supply probiotic
54	Guslandi M, Mezzi G, Sorghi M, Testoni PA. Saccharomyces boulardii in maintenance treatment of Crohn's disease. Dig Dis Sci 2000; 45: 1462-4.	No arthralgia component considered
55	Hart AL, Stagg AJ, Frame M, Graffner H, Glise H, Falk P, et al. The role of the gut flora in health and disease, and its modification as therapy. Aliment Pharmacol Ther. 2002;16(8):1383-93.	Discussion- no supply probiotic
56	Hart AL, Stagg AJ, Frame M, Graffner H, Glise H, Falk P, et al. Review article: The role of the gut flora in health and disease, and its modification as therapy. Alimentary Pharmacology and Therapeutics. 2002;16(8):1383-93.	Discussion- no supply probiotic
57	Heeney DD, Gareau MG, Marco ML. Intestinal Lactobacillus in health and disease, a driver or just along for the ride? Current Opinion in Biotechnology. 2018;49:140-7.	Discussion- no supply probiotic
58	Jethwa H, Abraham S. The evidence for microbiome manipulation in inflammatory arthritis. Rheumatology (Oxford). 2017;56(9):1452-60.	Discussion- no supply probiotic
59	Kang Y, Cai Y, Zhang X, Kong X, Su J. Altered gut microbiota in RA: implications for treatment. Zeitschrift fur Rheumatologie. 2017;76(5):451-7.	Discussion- no supply probiotic
60	Karimi O, Pena AS. Probiotics in arthralgia and spondyloarthropathies in patients with inflammatory bowel disease. Prospective randomized trials are necessary. Rev Esp Enferm Dig. 2005;97(8):570-4.	Discussion- no supply probiotic

61	Karimi O, Pena AS. Indications and challenges of probiotics, prebiotics, and synbiotics in the management of arthralgias and spondyloarthropathies in inflammatory bowel disease. J Clin Gastroenterol. 2008;42 Suppl 3 Pt 1:S136-41.	Discussion- no supply probiotic
62	Karimi O, Pena AS, van Bodegraven AA. Probiotics (VSL#3) in arthralgia in patients with ulcerative colitis and Crohn's disease: a pilot study. Drugs Today (Barc). 2005;41(7):453-9.	active inflammatory arthritis excluded
63	Keeney KM, Yurist-Doutsch S, Arrieta MC, Finlay BB. Effects of antibiotics on human microbiota and subsequent disease. Annu Rev Microbiol. 2014;68:217-35.	Discussion- no supply probiotic
64	Kelly D, Mulder IE. Microbiome and immunological interactions. Nutrition Reviews. 2012;70(SUPPL. 1):S18-S30.	Discussion- no supply probiotic
65	Keshwani A, Malhotra B, Kharkwal H. Nutraceutical: A drug, dietary supplement, and food ingredient. Current Pharmacogenomics and Personalized Medicine. 2015;13(1):14-22.	Discussion- no supply probiotic
66	Keyser FD, Mielants H, Veys EM. Current use of biologicals for the treatment of spondyloarthropathies. Expert Opin Pharmacother 2001 Jan; 2 (1): 85-93	Not including probiotics
67	Kim D, Zeng MY, Nunez G. The interplay between host immune cells and gut microbiota in chronic inflammatory diseases. Exp Mol Med. 2017;49(5):e339.	Discussion- no supply probiotic
68	Lomax AR, Calder PC. Probiotics, immune function, infection and inflammation: a review of the evidence from studies conducted in humans. Curr Pharm Des. 2009;15(13):1428-518.	Discussion- no supply probiotic
69	Lukaczer D. An integrative nutritional approach to the treatment of rheumatoid arthritis. Integrative Medicine: A Clinician's Journal. 2005;4(2):16-22.	No usable outcomes
70	Malchow HA. Crohn's disease and Escherichia coli: a new approach in therapy to maintain remission of colonic Crohn's disease? J Clin Gas-troenterol 1997; 25:653-8.	out of timeline
71	Mattila-Sandholm T, Blum S, Collins JK, et al. Probiotics: towards demonstrating efficacy. Trends Food Sci Technol 1999; 10:393-9.	out of timeline
72	Mahajan B, Singh V. Recent trends in probiotics and health management: A review. International Journal of Pharmaceutical Sciences and Research. 2014;5(5):1643-52.	Discussion- no supply probiotic
73	Manasson J, Scher JU. Spondyloarthritis and the microbiome: new insights from an ancient hypothesis. Curr Rheumatol Rep. 2015;17(2):10.	Discussion- no supply probiotic
74	McKean J, Naug H, Nikbakht E, Amiet B, Colson N. Probiotics and Subclinical Psychological Symptoms in Healthy Participants: A Systematic Review and Meta-Analysis. Journal of Alternative and Complementary Medicine. 2017;23(4):249-58.	not Inflammatory Arthritis
75	Mohammed AT, Khattab M, Ahmed AM, Turk T, Sakr N, A MK, et al. The therapeutic effect of probiotics on rheumatoid arthritis: a systematic review and meta-analysis of randomized control trials. Clinical Rheumatology. 2017;36(12):2697-707.	Systematic review
76	Mohsen Mazidi 1,2 , Peyman Rezaie 3 , Gordon A. Ferns 4 and Hassan Vatanparas Impact of Probiotic Administration on Serum C-Reactive Protein Concentrations: Systematic Review and Meta-Analysis of Randomized control trials	Systematic review
77	Montrose DC, Floch MH. Probiotics used in human studies. Journal of Clinical Gastroenterology. 2005;39(6):469-84.	Systematic review
78	Moriarty B, Bourke JF, Groeger D, Wycherly C, O'Mahony L, Malik M, et al. Bifidobacterium infantis 35624 in patients with mild to moderate chronic plaque psoriasis: A pilot study. British Journal of Dermatology. 2012;167(6):e31.	not Inflammatory Arthritis
79	Moriarty B, Groeger D, Wycherley C, O'Mahony L, Malik M, Whately-Smith C, et al. Bifidobacterium longum subspecies infantis	not Inflammatory Arthritis

	35624 in patients with mild to moderate chronic plaque psoriasis: A pilot study. British Journal of Dermatology. 2012;167:47.	
80	Naidoo K, Gordon M, Fagbemi AO, Thomas AG, Akobeng AK. Probiotics for maintenance of remission in ulcerative colitis. Cochrane Database Syst Rev. 2011(12):CD007443.	not Inflammatory Arthritis
81	Neville BA, Otoole P. Probiotic properties of Lactobacillus salivarius and closely related ctobacillus species. Future Microbiology. 2010;5(5):759-74.	not Inflammatory Arthritis
82	Nousiainen P, Merras-Salmio L, Aalto K, Kolho KL. Complementary and alternative medicine use in adolescents with inflammatory bowel disease and juvenile idiopathic arthritis. BMC Complement Altern Med. 2014;14:124.	Survey of use - no supply of probiotics
83	O'Hara AM, Shanahan F. Mechanisms of action of probiotics in intestinal diseases. TheScientificWorldJournal. 2007;7:31-46.	Discussion- no supply probiotic
84	Parvez S, Malik KA, Ah Kang S, Kim HY. Probiotics and their fermented food products are beneficial for health. Journal of Applied Microbiology. 2006;100(6):1171-85.	Discussion- no supply probiotic
85	Paulus L. Probiotics, saw palmetto, kampo and cannabis discussed in latest FACT. Pharmaceutical Journal. 2006;277(7418):347.	Discussion- no supply probiotic
86	Pan H, Li R, Li T, Wang J, Liu L. Whether probiotic supplementation benefits rheumatoid arthritis patients: a systematic review and meta-analysis. Engineering. 2017 Feb 1;3(1):115-21.	Systematic review
87	Presterl E, Kneifel W, Mayer HK, Zehetgruber M, Makristathis A, Graninger W. Endocarditis by Lactobacillus rhamnosus due to yogurt ingestion? Scand J Infect Dis. 2001;33(9):710-4.	not Inflammatory Arthritis
88	Reardon S. Microbiome therapy gains market traction. Nature. 2014;509(7500):269-70.	Discussion- no supply probiotic
89	Reimold AM, Chandran V. Nonpharmacologic therapies in spondyloarthritis. Best Pract Res Clin Rheumatol. 2014;28(5):779-92.	Discussion- no supply probiotic
90	Rodriguez-Castaño GP, Caro-Quintero A, Reyes A, Lizcano F. Advances in gut microbiome research, opening new strategies to cope with a western lifestyle. Frontiers in Genetics. 2017;7(JAN).	not Inflammatory Arthritis
91	Rohatgi S, Ahuja V, Makharia GK, Rai T, Das P, Dattagupta S, et al. VSL#3 induces and maintains shortterm clinical response in patients with active microscopic colitis: a two-phase randomised clinical trial. BMJ Open Gastroenterology. 2015;2(1).	not Inflammatory Arthritis
92	Rovensky J, Stancikova M, Svik K, Uteseny J, Bauerova K, Jurcovicova J. Treatment of adjuvant-induced arthritis with the combination of methotrexate and probiotic bacteria Escherichia coli O83 (Colinfant). Folia Microbiol (Praha). 2009;54(4):359-63.	Murine
93	Ruiz-Quezada SL, Martínez-Bonilla GE, De La Cruz-Castro AA, Estrada-Martínez KP, González-Diaz V, Castãeda Urea M, et al. Effect of probiotic lactobacillus casei shirota on clinical manifestations and serum cytokines in patients with rheumatoid arthritis. Annals of the Rheumatic Disease. 2013;71.	abstract only
94	Saarela M, Lähteenmäki L, Crittenden R, Salminen S, Mattila-Sandholm T. Gut bacteria and health foods - The European perspective. International Journal of Food Microbiology. 2002;78(1-2):99-117.	Discussion- no supply probiotic
95	Sanders ME. Considerations for use of probiotic bacteria to modulate human health. Journal of Nutrition. 2000;130(2 SUPPL.):384S-90S.	Discussion- no supply probiotic
96	Sandhya P, Danda D, Sharma D, Scaria V. Does the buck stop with the bugs?: an overview of microbial dysbiosis in rheumatoid arthritis. Int J Rheum Dis. 2016;19(1):8-20.	Discussion- no supply probiotic
97	Sanges M, Valente G, Rea M, Della Gatta R, De Franchis G, Sollazzo R, et al. Probiotics in spondyloarthropathy associated with ulcerative colitis: a pilot study. Eur Rev Med Pharmacol Sci. 2009;13(3):233-4.	abstract only

98	Saxelin M, Tynkkynen S, Mattila-Sandholm T, De Vos WM. Probiotic and other functional microbes: From markets to mechanisms. Current Opinion in Biotechnology. 2005;16(2):204-11.	Discussion- no supply probiotic
99	Schorpion A, Kolasinski SL. Can Probiotic Supplements Improve Outcomes in Rheumatoid Arthritis? Current Rheumatology Reports. 2017;19(11).	Discussion- no supply probiotic
100	Semerano L, Julia C, Aitisha O, Boissier MC. Nutrition and chronic inflammatory rheumatic disease. Joint Bone Spine. 2017;84(5):547-52.	Discussion- no supply probiotic
101	Sharpe PA, Wilcox S, Schoffman DE, Hutto B, Ortaglia A. Association of complementary and alternative medicine use with symptoms and physical functional performance among adults with arthritis. Disabil Health J. 2016;9(1):37-45.	Not probiotic
102	Sheikhi A, Nazarian M, Khadem-Al-Melleh A, Nasab NM, Esmaeilzadeh A, Yahaghi N, et al. In-vitro effects of Mycobacterium bovis BCG-lysate and its derived heat shock proteins on cytokines secretion by blood mononuclear cells of rheumatoid arthritis patients in comparison with healthy controls. Int Immunopharmacol. 2008;8(6):887-92.	Not live
103	Singh DD, Amdekar S, Singh V. Probiotics: Defenders of gastrointestinal habitats. Gastroenterology Insights. 2012;4(2):90-104.	Discussion- no supply probiotic
104	Singh P, Rani B, Chauhan AK, Maheshwari R. Healthy living with nutraceuticals. International Research Journal of Pharmacy. 2011;2(12):12-4.	Not probiotic
105	Slingerland AE, Schwabkey Z, Wiesnoski DH, Jenq RR. Clinical evidence for the microbiome in inflammatory diseases. Frontiers in Immunology. 2017;8(APR).	Discussion- no supply probiotic
106	Snydman DR. The safety of probiotics. Clinical Infectious Diseases. 2008;46(SUPPL. 2):S104-S11.	Discussion- no supply probiotic
107	Song SC, An YM, Shin JH, Chung MJ, Seo JG, Kim E. Beneficial effects of a probiotic blend on gastrointestinal side effects induced by leflunomide and amlodipine in a rat model. Beneficial Microbes. 2017;8(5):801-8.	Murine
108	Stebbings SM, Taylor C, Tannock GW, Baird MA, Highton J. The immune response to autologous bacteroides in ankylosing spondylitis is characterized by reduced interleukin 10 production. J Rheumatol. 2009;36(4):797-800.	Discussion- no supply probiotic
109	Steves CJ, Bird S, Williams FM, Spector TD. The Microbiome and Musculoskeletal Conditions of Aging: A Review of Evidence for Impact and Potential Therapeutics. J Bone Miner Res. 2016;31(2):261-9.	Discussion- no supply probiotic
110	Stoll ML, Cron RQ. The microbiota in pediatric rheumatic disease: epiphenomenon or therapeutic target? Curr Opin Rheumatol. 2016;28(5):537-43.	Discussion- no supply probiotic
111	Thygesen JB, Glerup H, Tarp B. Saccharomyces boulardii fungemia caused by treatment with a probioticum. BMJ Case Rep. 2012;2012.	not Inflammatory Arthritis
112	Tlaskalova-Hogenova H, Stepankova R, Hudcovic T, Tuckova L, Cukrowska B, Lodinova-Zadnikova R, et al. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. Immunol Lett. 2004;93(2-3):97-108.	Discussion- no supply probiotic
113	Tlaskalova-Hogenova H, Stepankova R, Hudcovic T, Tuckova L, Cukrowska B, Lodinova-Zadnikova R, et al. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. Immunol Lett. 2004;93(2-3):97-108.	Discussion- no supply probiotic
114	Tournadre A, Tatar Z, Coxam V, Soubrier M. Gut microbiota and diet in rheumatoid arthritis. Revue du Rhumatisme Monographies. 2018;85(1):52-6.	Discussion- no supply probiotic
115	Toussirot E, Robinet E, Saas P, Chabod J, Auge B, Cozma G, et al. Bacterial extract (OM-89) specific and non specific	Not live

	immunomodulation in rheumatoid arthritis patients. Autoimmunity. 2006;39(4):299-306.	
116	Toussirot É, Robinet É, Saas P, Chabod J, Augé B, Cozma G, et al. Bacterial extract (OM-89) specific and non specific immunomodulation in rheumatoid arthritis patients. Autoimmunity. 2006;39(4):299-306.	Not live
117	Toussirot EA. Oral tolerance in the treatment of rheumatoid arthritis. Curr Drug Targets Inflamm Allergy. 2002;1(1):45-52.	Not probiotic
118	Vaghef-Mehrabany E, Homayouni-Rad A, Alipour B, Sharif SK, Vaghef-Mehrabany L, Alipour-Ajiry S. Effects of Probiotic Supplementation on Oxidative Stress Indices in Women with Rheumatoid Arthritis: A Randomized Double-Blind Clinical Trial. J Am Coll Nutr. 2016;35(4):291-9.	Outcome exclusion
119	Van Bodegraven AA, Karimi O, Peña AS. IBD-related spondylarthropathy and probiotics. EOS Rivista di Immunologia ed Immunofarmacologia. 2004;24(1-2):52-5.	Unable to obtain in English
120	Van de Wiele T, Van Praet JT, Marzorati M, Drennan MB, Elewaut D. How the microbiota shapes rheumatic diseases. Nat Rev Rheumatol. 2016;12(7):398-411.	Discussion- no supply probiotic
121	Van den Nieuwboer M, Brummer RJ, Guarner F, Morelli L, Cabana M, Claassen E. The administration of probiotics and synbiotics in immune compromised adults: Is it safe? Beneficial Microbes. 2015;6(1):3-17.	Discussion- no supply probiotic
122	van der Meulen TA, Harmsen H, Bootsma H, Spijkervet F, Kroese F, Vissink A. The microbiome-systemic diseases connection. Oral Dis. 2016;22(8):719-34.	Discussion- no supply probiotic
123	Vanderhoof JA, Young RJ. Use of probiotics in childhood gastrointestinal disorders. Journal of Pediatric Gastroenterology and Nutrition. 1998;27(3):323-32.	Discussion- no supply probiotic
124	Vanderhoof JA, Young RJ. Current and potential uses of probiotics. Annals of Allergy, Asthma and Immunology. 2004;93(5 SUPPL.):S33-S7.	Discussion- no supply probiotic
125	Vitaliti G, Pavone P, Guglielmo F, Spataro G, Falsaperla R. The immunomodulatory effect of probiotics beyond atopy: an update. J Asthma. 2014;51(3):320-32.	Discussion- no supply probiotic
126	Vitetta L, Cicuttini F, Sali A. Alternative therapies for musculoskeletal conditions. Best Pract Res Clin Rheumatol. 2008;22(3):499-522.	Discussion- no supply probiotic
127	Vitetta L, Coulson S, Thomsen M, Nguyen T, Hall S. Probiotics, D–Lactic acidosis, oxidative stress and strain specificity. Gut Microbes. 2017;8(4):311-22.	not Inflammatory Arthritis
128	Vitetta L, Manuel R, Zhou JY, Linnane AW, Hall S, Coulson S. The overarching influence of the gut microbiome on end-organ function: The role of live probiotic cultures. Pharmaceuticals. 2014;7(9):954-89.	Discussion- no supply probiotic
129	Wang H, Lee IS, Braun C, Enck P. Effect of probiotics on central nervous system functions in animals and humans: A systematic review. Journal of Neurogastroenterology and Motility. 2016;22(4):589-605.	not Inflammatory Arthritis
130	Wang P, Tao JH, Pan HF. Probiotic bacteria: a viable adjuvant therapy for relieving symptoms of rheumatoid arthritis. Inflammopharmacology. 2016;24(5):189-96.	Discussion- no supply probiotic
131	Wędrychowicz A, Zając A, Tomasik P. Advances in nutritional therapy in inflammatory bowel diseases: Review. World Journal of Gastroenterology. 2016;22(3):1045-66.	not Inflammatory Arthritis
132	Wendling D. The gut in spondyloarthritis. Joint Bone Spine. 2016;83(4):401-5.	Discussion- no supply probiotic
133	Wendling D, Vuitton L, Koch S, Prati C. Spondyloarthritis and the gut: a new look. Joint Bone Spine. 2015;82(2):77-9.	Discussion- no supply probiotic
134	Wu X, He B, Liu J, Feng H, Ma Y, Li D, et al. Molecular Insight into Gut Microbiota and Rheumatoid Arthritis. Int J Mol Sci. 2016;17(3):431.	Discussion- no supply probiotic

135	Xu Y, Liu Y, Liu Y, Pei J, Yao S, Cheng C. Bacteriophage therapy against Enterobacteriaceae. Virologica Sinica. 2015;30(1):11-8.	Discussion- no supply probiotic
136	Xu YY, Tan X, He YT, Zhou YY, He XH, Huang RY. Role of gut microbiome in ankylosing spondylitis: an analysis of studies in literature. Discov Med. 2016;22(123):361-70.	Discussion- no supply probiotic
137	Yan F, Polk DB. Commensal bacteria in the gut: Learning who our friends are. Current Opinion in Gastroenterology. 2004;20(6):565-71.	Discussion- no supply probiotic
138	Yang L, Wang L, Wang X, Xian CJ, Lu H. A Possible Role of Intestinal Microbiota in the Pathogenesis of Ankylosing Spondylitis. Int J Mol Sci. 2016;17(12).	Discussion- no supply probiotic
139	Yeoh N, Burton JP, Suppiah P, Reid G, Stebbings S. The role of the microbiome in rheumatic diseases. Curr Rheumatol Rep. 2013;15(3):314.	Discussion- no supply probiotic
140	Zashin SJ. Complementary and alternative therapies for arthritis: science or fiction? Journal of Musculoskeletal Medicine. 2000;17(6):330-45.	Discussion- no supply probiotic
141	Zhang F, Zhu H. Reply to Jia. American Journal of Gastroenterology. 2015;110(12):1731-2.	Discussion- no supply probiotic
142	Zhong D, Wu C, Zeng X, Wang Q. The role of gut microbiota in the pathogenesis of rheumatic diseases. Clinical Rheumatology. 2017:1-10.	Discussion- no supply probiotic