Probiotics and Health

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EXECUTIVE SUMMARY

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Although certain effects can be ascribed to probiotics as a general class, including their contribution to a healthy gut microbiotia, supporting a healthy digestive tract and a healthy immune system, many effects of probiotics are strain-specific. This means that if a certain health effect is found for one strain, no conclusions can be made on the effect of another strain, and vice versa. Probiotics do not become established members of the intestinal gut flora, so for them to confer a long-lasting benefit, they must be consumed regularly. There has been vast interest in the health benefits, which is reflected in the large number of studies now published in this field. This paper aims to consider the evidence on the effects of probiotics on health, with a focus on well-studied areas.

Probiotics have been widely studied in people suffering from irritable bowel syndrome (IBS) since a role of the microbiota in the gastrointestinal (GI) tract has been implicated. Evidence from Randomised Controlled Trials (RCTs) suggests use of probiotics is associated with a significant reduction in the risk of symptom persistence and also significantly reduces global IBS symptoms, abdominal pain, bloating and flatulence. Combinations of probiotics are generally effective, while the role of single *Lactobacillus (L.)* and *Bifidobacterium (B.)* strains is unclear, possibly due to a limited number of studies. In children with IBS, *L. rhamnosus* GG is associated with a significantly higher rate of treatment responders and reduced frequency of pain, although other strains and combinations may be effective too. Overall, the evidence suggests that IBS sufferers may benefit from taking probiotics.

The use of probiotics is promising for the treatment of constipation in adults by increasing stool frequency and consistency. On average, bowel movement frequency increased by 1.3 bowel movements per week. Studies using *B. lactis* were effective, while *L. casei* Shirota was not. Probiotics have to date failed to convey a benefit in constipated children.

Inflammatory bowel disease (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD), greatly affects quality of life of those affected by it. It has been suggested an altered gut flora has a role to play in the development and aggravation of IBD. Probiotics have been found effective in the management of UC. The strain *Escherichia (E.) coli* Nissle 1917 was as effective as the gold standard treatment mesalazine in maintaining remission, while the multi-strain probiotic VSL#3 taken with medication was significantly superior to placebo in improving UC disease activity. For other strains or combinations of strains the evidence is to date limited. UC sufferers who undergo surgery have a high chance of developing an infection of the artificial pouch which replaces the colon (pouchitis). The probiotic combination VSL#3 was found to be effective in maintaining remission in those suffering from chronic pouchitis, and also in lowering the risk of developing pouchitis in the first place. Evidence to date does not suggest that probiotics are effective in the treatment and management of CD.

A large number of studies have investigated the effect of probiotics in preventing antibiotic-associated diarrhoea (AAD). Overall, probiotics are effective in reducing the risk of AAD in both children and adults by around 42-48%, but may not be effective in older adults. There was no evidence the effectiveness of probiotic interventions varied between different types of probiotics, although strains used were often not well documented. A number of studies investigated a specific type of AAD caused by the toxigenic bacteria *Clostridium (C.) difficile*. Systematic reviews and meta-analyses have found probiotics can lower the risk of *C. difficile*-associated diarrhoea (CDAD) by more than 60% in children and adults (outpatients), and in adult inpatients. In one of the reviews probiotics were associated with a lower risk of CDAD but not with a lower occurrence of positive stool cytotoxin/culture for *C. difficile*, suggesting probiotics may be effective in preventing symptoms of infection or limiting the extent of infection rather than inhibiting the colonisation of *C. difficile* itself. One recent large multicentre trial

from the UK (not included in above mentioned reviews) did not find a beneficial effect of a combination of two Bificobacteria and two Lactobacilli strains in hospitalised patients, although the occurrence of CDAD was generally very low in this study (clearly lower than in other studies).

Acute diarrhoea is a leading cause of childhood morbidity and an important cause of malnutrition. Numerous studies have investigated the use of probiotics in treating acute diarrhoea, and overall were found to be effective and shorten the duration of diarrhoea by an average of one full day. The most studied probiotics are L. Rhamnosus GG, Saccharomyces (S.) boulardii and Enterococcus lactic acid bacteria SF68, which were all effective. A limited number of studies have investigated the effectiveness of probiotics in treating persistent diarrhoea (>14 days), with evidence suggesting probiotics may be effective in shortening duration.

Probiotics have the potential to regulate critical components of the immune system, thereby lowering the risk of infections such as respiratory infections, including the common cold. A review found that on average use of probiotics reduced the number of patients experiencing episodes of upper respiratory tract infections by around 47% and the duration of an episode by almost 2 days. However, these findings need to be interpreted with caution due to the overall low quality and risk of bias of the included studies.

The use of probiotics has also been studied in relation to allergies, in particular relating to atopic dermatitis (or eczema) and asthma. Evidence to date suggests that use of probiotics during pregnancy and during pregnancy and infancy, but not during infancy alone, is associated with a reduced risk of atopic dermatitis. The most commonly studied strain is *L. rhamnosus* GG, which proved effective in lowering the risk of atopic dermatitis, as were combinations of probiotic strains. There is some evidence to suggest that Lactobacillus strains, but not Bifidobacterium strains, are effective in treating atopic disease in children, although the observed effect was small and possibly clinically insignificant. Evidence to date does not suggest that probiotic use during pregnancy and/or infancy reduces the risk of wheeze and asthma.

Probiotics, as well as prebiotics, are increasingly used in infant formulae in an attempt to promote a gut microflora resembling more closely that of breastfed infants. While probiotics are considered safe to use in infant formulae, evidence to date is considered too limited to draw reliable conclusions. However, the supplementation of formula with probiotics is considered an important field of research, and well-designed RCTs are needed to investigate the effect of probiotics on growth, GI infections, respiratory symptoms, colic and crying.

There is emerging evidence for a role of probiotics in treatment of obesity and for bone health, but more evidence is needed before any conclusions can be drawn.

Use of probiotics is generally considered safe for the general population, although a more systematic approach to monitor adverse events is warranted.

Overall, evidence suggests that there is a clear benefit of taking probiotics in order to prevent or treat certain health issues. A diversity of strains have been studied, making it difficult to identify which specific strains are effective, although for certain areas such as AAD a variety of strains seem to be effective. More research is needed to identify whether and how healthy consumers may benefit from taking probiotics.

BACKGROUND AND DEFINITIONS

Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit to the host' (FAO/WHO 2002). This working definition by the Food and Agriculture Organization of the United Nations and the World Health Organization has become the mostly widely adopted and accepted definition worldwide. Although it has been suggested dead bacteria products derived from bacteria or end products of bacterial growth may also impart some health benefits, these are not considered probiotics because they are not alive when administered (Hill *et al.* 2014).

Studies have shown that the many effects of probiotics are strain-specific, which means if a specific health effect is found on administration of one specific strain, no conclusions can be drawn on the effectiveness of other strains (Weichselbaum 2009). However, an expert panel convened by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2013 came to the conclusion that certain effects can be ascribed to probiotics as a general class, which is in line with regulatory approaches in Canada and Italy. For example, Health Canada has accepted certain species belonging to the genus *Lactobacillus* (*acidophilus, casei, fermentum, gasseri, johnsonii, paracasei, plantarum, rhamnosus* and *salivarius*) and *Bifidobacerium* (*adolescentis, animalis, bifidum, breve* and *longum*) as probiotics, for which non strain-specific claims might be made, provided they are delivered in food at a level of $1x10^9$ (one billion) colony forming units (CFU) per serving (Health Canada 2009). These general acceptable claims mostly are based on their contribution to a healthy gut microbiota (although the current state of science does not allow a clear definition of a healthy gut microbiota based on microbial composition). Experts believe the general benefit of probiotics on gut microbiota derives from creating a more favourable gut environment, through mechanisms shared by most probiotics. Further common general benefits often associated with probiotics are supporting a healthy digestive tract and a healthy immune system (Hill *et al.* 2014). Although general health benefits seem to be supported by probiotics overall, for more specific health benefits it is important to study each strain (or a combination) separately for their efficacy.

Probiotic strains do not become established members of the intestinal flora but generally persist only for the period of consumption and for a relatively short period thereafter (Corthésy *et al.* 2007). This means that for probiotics to confer a long-lasting benefit, they must be consumed regularly. Probiotics are usually administered orally and are available in various forms, including food products (e.g. dairy food), capsules, sachets or tablets. The choice of the format has much to do with personal preference and individual needs, but also shelf life and convenience (Weichselbaum 2009).

There has been vast interest in the health benefits of probiotics, which is reflected in the large number of studies now published in this field. The aim of this paper is to consider the current evidence on the effects of probiotics on health, focusing mostly on well-

Abbreviations:

L.	*Lactobacillus*
B.	*Bifidobacterium*
S.	*Saccharomyces*
E.	*Escherichia*
C.	*Clostridium*
CFU	*Colony-forming units*
IBD	*Inflammatory bowel disease*
CD	*Crohn's disease*
UC	*Ulcerative colitis*
IBS	*Irritable bowel syndrome*
AAD	*Antibiotic-associated diarrhoea*
CDAD	*C. difficile-associated diarrhoea*
RCT	*Randomised controlled trial*
RR	*Relative risk*
GI	*Gastrointestinal*

studied areas, while emerging areas are also briefly discussed. The objective is to provide a clearer picture of whether and how probiotics can be beneficial for health. Prebiotics are out of scope of this review.

FUNCTIONAL GASTROINTESTINAL DISORDERS

Functional gastrointestinal (GI) disorders are a variable combination of GI symptoms not explained by structural or biochemical abnormalities. They include abdominal pain-related disorders (including irritable bowel syndrome [IBS]) and functional constipation (Horvath & Szajewska 2013).

IRRITABLE BOWEL SYNDROME

IBS is characterised by intermittent abdominal pain, altered bowel habits (diarrhoea and/or constipation) and other GI symptoms including flatulence and bloating in the absence of structural abnormalities (Cremonini & Talley 2005). People with IBS commonly report incomplete evacuation/rectal hypersensitivity, as well as urgency, which is increased in diarrhoea-predominant IBS (NICE 2008). An estimated 10-20% of people in developed countries are affected, making IBS a problematic disorder resulting in impaired quality of life for patients and high utilisation of healthcare services. The pathophysiology of IBS is multifactorial and a role of the microbiota in the GI tract has been implicated, with studies showing that many IBS sufferers have lower Lactobacilli and Bifidobacteria (Whelan 2011).

Numerous randomised controlled trials (RCTs) have been carried out investigating the use of probiotics in the management of IBS, although few used the same probiotic strains. Several systematic reviews have found a beneficial effect of probiotics on global IBS symptoms, abdominal pain and flatulence (see Whelan 2011). A recently published systematic review and meta-analysis is the largest synthesis of data in IBS patients so far and includes 35 relevant RCTs involving 3,452 patients (Ford *et al.* 2014). The paper confirmed findings from earlier reviews. Overall, the use of probiotics resulted in a significant reduction in the risk of symptom persistence by around 21%, which was statistically significant but significant heterogeneity was detected between studies. This translates in a number needed to treat of seven to prevent symptom persistence in one patient. Many of the studies included in the meta-analysis had an unknown risk of bias, which means the true effect may be overestimated. However, when only including studies that had low risk of bias, the effect of probiotics was still statistically significant with an 18% risk reduction (Ford *et al.* 2014).

More detailed analysis revealed that combination probiotics, used in 12 RCTs, led to a significant effect on symptoms persistence, but again with significant heterogeneity. Three trials used the same combination of *L. paracasei* ssp *paracasei* F19, *L. acidophilus* La5, and *B. lactis* Bb12, with no benefit over placebo. Probiotics of the *Lactobacillus* species were used in six trials, again with no clear benefit but also significant heterogeneity. Three RCTs that used one specific *Lactobacillus* strain, *L. plantarum* DSM 9843, found a significantly lower risk of symptom persistence by 33%, although again significant heterogeneity was observed (Ford *et al.* 2014).

As well as reducing the risk of symptoms persistence, probiotics also significantly reduced global IBS symptoms or abdominal pain, as well as bloating and flatulence, while there was no apparent effect on urgency symptoms. Further analysis found studies using combinations of probiotics significantly improved IBS symptom scores, while studies using *Lactobacillus* or *Bifidobacterium* strains resulted in no statistically significant benefit. The risk of adverse events was higher in those taking probiotics with 16.5% experiencing adverse events, compared to 13.8% of those assigned to placebo (Ford *et al.* 2014).

Overall, probiotics reduce the risk of symptom persistence and of symptom scores in IBS sufferers. However, different probiotic species/strains have different microbiological characteristics that will likely affect their clinical efficacy in IBS, i.e. not all probiotics will improve symptoms. This was likely partly responsible for the heterogeneity between studies and makes generalising the findings difficult (Whelan 2014). Combinations of probiotics seem effective, while for some specific probiotic strains no significant effect was found, which could possibly be due to the small number of studies for single strains and consequently insufficient power to detect any meaningful effect (Ford *et al.* 2014).

A meta-analysis investigating the effect of one specific probiotic strain, *L. rhamnosus* GG, in children with abdominal pain-related functional GI disorders including IBS found supplementation with the probiotic was associated with a significantly higher rate of treatment responders (no pain or decrease in pain intensity) in the overall study population, and also in the IBS group specifically. The intensity of pain was significantly reduced in the overall study population and in the IBS subgroup, while frequency of pain was reduced in the IBS subgroup only (Horvath *et al.* 2011). A more recent meta-analysis in children with abdominal pain-related functional GI disorders also included studies using other probiotics including *L. reuteri* DSM 17 938 and the combination product VSL#3. Overall, use of probiotics led to a significantly higher treatment success rate compared to placebo, with the likelihood of treatment success (no abdominal pain or reduction in pain) being around 50% higher with probiotic use. There was no significant heterogeneity. Sub-group analysis showed the treatment effect was only significant in IBS sufferers, who had a 62% higher likelihood of treatment success compared to placebo (Korterink *et al.* 2013).

Some IBS studies have found a relatively high placebo response, which could be attributed to the fact that assessment of IBS mostly relies on subjective estimations of symptoms. This makes identifying the real effect of probiotics challenging (Weichselbaum 2009).

- Probiotics seem more effective than placebo in reducing symptoms in IBS sufferers, in both adults and children. Combination probiotics seem effective, while no significant effect for the use of single strains (or genera) was found. However, this could be due to the small number of studies using single strains with insufficient power to detect any meaningful effect.
- Overall, the evidence to date suggests IBS sufferers may benefit from using probiotics.

CONSTIPATION

Constipation is widespread and greatly affects patients' quality of life. A significant proportion of people with constipation are dissatisfied with pharmacological treatments (Whelan 2014).

It has been suggested probiotics may benefit functional constipation in several ways, including modification of the GI microbiota, which is known to be altered in constipation; alteration of gut function through probiotic metabolites; and increased production of lactate and short-chain fatty acids, reducing luminal pH, which some researchers have proposed will enhance gut transit time (Dimidi *et al.* 2014). A recent systematic review and meta-analysis included findings from 14 RCTs in 1182 adults who had functional constipation but were otherwise healthy (e.g. no IBS). The most commonly studied species/strains were *B. lactis* (7 studies) and *L. casei* Shirota (4 studies), while for other strains only single studies were available. Meta-analysis of data from ten trials found probiotics significantly increased stool frequency by 1.3 bowel movements per week ($p<0.0001$), although there was significant heterogeneity. Further analysis showed that *B. lactis* significantly increased stool frequency by 1.5 bowel movements per week ($p=0.0003$), again with significant heterogeneity, while *L. casei* Shirota did not significantly affect stool frequency and there was no heterogeneity. The study authors suggested that probiotics, in particular of the species *B. lactis*, have at least half of the efficacy of laxatives in increasing stool frequency. Use of probiotics also led to improved stool consistency

compared to placebo, which means stools were becoming less hard/more soft, but there was significant heterogeneity. *B. lactis* significantly improved stool consistency while *L. casei* Shirota had no effect, both with significant heterogeneity. Stool quantity was not affected by use of probiotics (Dimidi *et al.* 2014).

Probiotics also significantly improved bloating in patients with constipation, significantly reduced sensation of incomplete evacuation and significantly reduced the occurrence of hard stools (all with significant heterogeneity), while no influence on flatulence was found. Five of the six studies that measured adverse events reported none occurred in either the probiotic or the placebo group (Dimidi *et al.* 2014).

Dimidi and colleagues also found that probiotics significantly reduced gut transit time by half a day, although this was based on only 2 studies with 3 intervention arms. Nonetheless, another meta-analysis also showed a significant decrease in gut transit time, albeit in a mixed population of healthy people and people with constipation and constipation-predominant IBS. In particular the two strains *B. lactis* HN019 and *B. lactis* DN-173 010 showed a medium to large treatment effect, while treatment effects with other single strains and combination products were small and not statistically significant (Miller & Ouwehand 2013). The results of the meta-analysis by Dimidi *et al*. (2014) are based on short-term administration of probiotics (2-8 weeks) as no studies with long-term use are available.

Another systematic review and meta-analysis found no evidence showing probiotics are more effective than placebo regarding treatment outcome or increasing defecation frequency in constipated children (Korterink *et al.* 2013).

- Probiotics seem effective in treatment of constipation in adults by increasing stool frequency and consistency. Further investigation found that probiotics of the species *B. lactis* were effective, while *L. casei* Shirota failed to improve symptoms of constipation. Probiotics have to date failed to convey a benefit in constipated children.

INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) comprises different conditions of the gut, of which the two main types are ulcerative colitis (UC) and Crohn's disease (CD). While UC is limited to the colon and rectum, CD may affect any part of the GI tract, most commonly the lower small intestine (Carter *et al.* 2004). Both conditions greatly affect quality of life. Although the exact pathophysiology of IBD is unknown and is likely multifactorial, it has been suggested that an altered gut flora has a role to play in the development or aggravation of IBD (World Gastroenterology Organisation 2009).

ULCERATIVE COLITIS

Several double-blind RCTs have investigated probiotics as a means to treat patients with ulcerative colitis (UC). Three studies used the strain *Escherichia (E.) coli* Nissle 1917, two used the multi-strain probiotic VSL#3[1], and one used a combination of *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* BB-12. One single-blind RCT used a combination of *B. breve* Yakult, *B. bifidum* Yakult and *L. acidophilus*.

The three studies using the strain *E. coli* Nissle 1917 were all carried out in UC patients that were in remission, i.e. the disease was not active, and all studies compared the efficacy of the probiotic strain

[1] VSL#3 contains four strains of Lactobacilli (*L. casei, L. plantarum, L. acidophilus* and *L. delbrueckii* ssp. *bulgaricus*), three strains of Bifidobacteria (*B. longum, B. breve* and *B. infantis*) and one strain of *Streptococcus salivariuus* ssp. *thermophilus*.

with the gold standard treatment mesalazine (also often referred to as 5-aminosalicylic acid). In all three studies treatment with *E. coli* Nissle 1917 was as effective as treatment with mesalazine, i.e. there was no significant difference in patients in terms of relapse (meaning the disease has become active) between the two groups (Kruis *et al.* 1997, Rembacken *et al.* 1999, Kruis *et al.* 2004). In one unblinded pilot study in children and adolescents (11-18 years) suffering from UC the strain *E. coli* Nissle 1917 was also as effective as mesalazine in maintaining remission (Henker *et al.* 2008).

The two double-blind, multicentre RCTs investigating the efficacy of the probiotic combination VSL#3 were carried out in patients with mild-to-moderately active UC, one in India and one in Italy. In both studies patients continued taking their medication (although certain drugs were prohibited) and received either VSL#3 or placebo for 12 weeks (Sood *et al.* 2009) or for 8 weeks (Tursi *et al.* 2010). In both studies VSL#3 was significantly superior to placebo in improving the UC disease activity index by at least 50% after 6-8 weeks. Almost half of the patients in both studies achieved remission, compared to only 15.7% in placebo group in one study, and 32.4% in the second study (Sood *et al.* 2009; Tursi *et al.* 2010).

The combination *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12 failed to demonstrate an effect on maintenance of remission in patients with UC in one double-blind RCT (Wildt *et al.* 2011).

In a small single-blind RCT, the combination *B. breve* Yakult, *B. bifidum* Yakult and *L. acidophilus* was used for treatment of active UC and was given for a period of 12 weeks in addition to standard treatment. The response rate (defined as decrease in the clinical activity index score of at least three points from a maximum score of 21 = highest activity) was higher in the probiotic group (70%) compared to the placebo group (33%), although statistical significance was not achieved, probably due to the small number of subjects (n=19). There was no significant difference in the number of patients achieving remission (40% and 33%, respectively) (Kato *et al.* 2004). In another small unblinded study patients given the same probiotic combination in addition to standard treatment had a lower rate of exacerbation of UC compared to those on standard treatment only. However, the study was small and exacerbation was measured on the basis of self-reported clinical symptoms (Ishikawa *et al.* 2002).

- The use of probiotics in treatment and management of UC is promising. *E. coli* Nissle 1917 was found to be as effective as the gold standard treatment in maintaining remission, and the probiotic combination VSL#3 when given with standard treatment was significantly superior to placebo in improving the UC disease activity. For other strains or combinations of strains the evidence to date is limited.

POUCHITIS

Around 25-30% of patients suffering from UC have to undergo surgery, where the colon is removed and replaced by an artificial pouch. Around 40-60% of those who undergo this surgery develop an inflammation of the ileal pouch, called pouchitis, which is the most common long-term complication in patients undergoing surgery for UC. Although the causes are not fully understood, it is assumed that the microflora in the pouch plays a role in the abnormal mucosal immune response (Pardi & Sandborn 2006).

Two double blind RCTs examined the efficacy of the probiotic combination VSL#3 in the maintenance of remission in patients suffering from chronic relapsing pouchitis, and recurrent or refractory pouchitis (Gionchetti *et al.* 2000; Mimura *et al.* 2004). In both studies, significantly fewer patients who were in remission at study entry relapsed during the 9 and 12 months of treatment. While all patients receiving placebo relapsed in one study (all within 4 months), and all but one patient relapsed in the

second study, in both studies only 15% of patients receiving VSL#3 relapsed throughout the study period (Gionchetti *et al.* 2000; Mimura *et al.* 2004). In another study, the efficacy of VSL#3 in the prevention of onset of pouchitis during the first year after restoration of the faecal stream was examined. In this study, significantly fewer patients developed pouchitis in the probiotic group (10%) compared with the placebo group (40%) (Gionchetti *et al.* 2003). In an uncontrolled study, Gionchetti *et al.* (2007) found that VSL#3 was successful in treating mild active pouchitis after 4 weeks, with complete remission in almost 70% of patients.

In one double-blind RCT, *L. rhamnosus* GG failed to treat pouchitis, suggesting this probiotic strain is not effective as primary therapy for pouchitis (Kuisma *et al.* 2003).

- The probiotic combination VSL#3 was effective in reducing relapse rate in patients with chronic/recurring pouchitis in remission, was effective in reducing the risk of developing pouchitis following surgery for UC, and may be effective in treating mild active pouchitis. *L. rhamnosus* GG was not found to be effective in treating active pouchitis.

CROHN'S DISEASE

The use of probiotics in CD is less promising than it is for UC and pouchitis. Four double-blind RCTs examined the efficacy of probiotics in preventing recurrence of CD following surgery or non-surgical treatment. Two studies used *L. johnsonii* LA1 (Marteau *et al.* 2006; Van Gossum *et al.* 2007), one used the strain *L. rhamnosus* GG (Prantera *et al.* 2002), and one used the probiotic yeast *Saccharomyces (S.) boulardii* (Bourreille *et al.* 2013). In all four studies, there was no significant effect of the intervention on recurrence rate (following surgery, 3 studies) or in relapse rate (following remission through medical treatment). *S. boulardii* was found to be effective when given with standard treatment (mesalazine) in one study, although findings have to be interpreted with caution as this was a small, unblinded RCT (Guslandi *et al.* 2000).

One double-blind RCT using a synbiotic (i.e. a combination of pre- and probiotic) showed promising results. In this study *B. longum* was given together with Synergy 1 (inulin/oligofructose) to patients with active CD, in addition to their current medical treatment. In this study, daily synbiotic consumption of a 6 months period resulted in significant improvements in clinical symptoms and in histological scores. However, this was not associated with marked improvements in the way patients felt the disease impacted on their way of life, despite the fact that many of the synbiotic patients went into clinical remission (Steed *et al.* 2010). No conclusions can be drawn on the independent effect of the studied probiotic. Further studies are needed to see if synbiotics may be a useful option for treating CD patients.

- Evidence to date does not suggest that probiotics are effective in the treatment and management of CD.

ANTIBIOTIC-ASSOCIATED DIARRHOEA

Diarrhoea is a common side effect of treatment with antibiotics, with rates of antibiotic-associated diarrhoea (AAD) varying depending on the type of antibiotic used (McFarland 1998). About 10-20% of all AAD cases are positive for toxigenic *Clostridium (C.) difficile* (Högenauer *et al.* 1998).

In New Zealand and many other countries, *C. difficile* is the most common cause of diarrhoea in hospitalised patients, with up to 20% of hospitalised patients being colonised with *C. difficile*, but only a minority experience symptomatic disease. *C. difficile*-associated disease can range from mild diarrhoea to severe life-threatening infection. Patients with certain medical conditions – including

those exposed to antibiotics or chemotherapy – and the elderly are at greater risk of developing symptomatic disease. Almost all antibiotics can cause *C. difficile* infection (Ministry of Health 2013).

A relatively large body of evidence is available on the efficacy of probiotic supplementation on risk of AAD. One systematic review and meta-analysis of parallel randomised controlled (both placebo, no treatment, or a different probiotic or probiotic dose) trials, both blinded and unblinded, identified 82 eligible studies, 63 of which reported the number of participants with diarrhoea and were used to calculate pooled risk (Hempel *et al.* 2012). Probiotic use was associated with a significantly lower risk (by 42%) of developing diarrhoea compared with control, with a number needed to treat to prevent one case of AAD of 13. Similar risk reductions that were statistically significant were found when only pooling findings from 44 double-blind RCTs and when only pooling 12 RCTs that declared the funding source and claimed to be free of conflict of interest. There was no evidence that the effectiveness of probiotic interventions varied between different types of probiotics, although the strains used were poorly documented in most studies. Risk reduction was similar in children and adults, while a limited number of studies (n=3) in older adults age 65 years or over found no significant benefit of probiotic supplementation on risk of AAD (Hempel *et al.* 2012).

Another systematic review and meta-analysis only included double-blinded, placebo-controlled RCTs published as full text papers in English language (Videlock & Cremonini 2012). Overall 34 RCTs comprising 4138 patients met the inclusion criteria, ten in children and the remainder in adults, of which 14 had a low risk of bias, while in 10 studies the risk was unclear and 10 studies had a high risk of bias. Meta-analysis of data resulted in a significantly lower risk of AAD (by 47%), which corresponded to an average number needed to treat of 8. Risk reduction was similar in studies that included children or adults. Again, the preventive effect was consistent across the probiotic species administered (Videlock & Cremonini 2012).

A Cochrane review on probiotics and AAD in children also found a significantly reduced risk (by 48%) in those taking probiotics compared to the control group. The combined incidence of AAD in the probiotic group was 9% compared to 18% in the control group. High doses of probiotics (≥5 billion CFUs/day) seem more effective than low probiotic doses (<5 billion CFUs/day) (Johnston *et al.* 2011).

- Probiotics are effective in reducing the risk of AAD in both children and adults by around 42-48%, but may not be effective in older adults. There was no evidence that effects differed between different strains, although strains were often not well documented.

C. difficile-ASSOCIATED DIARRHOEA AND INFECTION

A recent Cochrane review investigated the efficacy and safety of probiotic use in preventing *C. difficile*-associated diarrhoea (CDAD) in adults (>18 years) and children (0-18 years). RCTs that compared probiotics vs. placebo, alternative prophylaxis, or no treatment for the prevention of CDAD in children and adults taking probiotics were included. Pooling data from 23 trials and 4213 participants resulted in a statistically significant 64% risk reduction of CDAD in those taking probiotics. The incidence of CDAD was 2.0% in the probiotic group compared to 5.5% in the placebo/no treatment control group. With respect to the incidence of the secondary outcome measure *C. difficile* infection (i.e. positive stool cytotoxin/culture for *C. difficile* without occurrence of diarrhoea) no significant effect of probiotic treatment was found. This finding suggests that probiotics may be effective in preventing symptoms of infection or in limiting the extent of infection rather than inhibiting the colonisation and infection of *C. difficile* itself (Goldenberg *et al.* 2013).

Another systematic review and meta-analysis investigated the efficacy of probiotics in preventing AAD and CDAD in adult inpatients. Overall 16 studies with 2434 patients were included, ten of which used

Lactobacillus-based probiotics, and 5 evaluated *S. boulardii*. Pooled analysis resulted in a significantly reduced risk of AAD (by 39%) with a number needed to treat to benefit of 11, and a significantly reduced risk (by 63%) of CDAD with a number to treat to benefit of 14. The incidence of CDAD was 3.1% in the intervention arm and 10.4% in the placebo arm. When only including high quality studies in the analysis, a significant 76% risk reduction was found for CDAD, while pooled analysis of poor and fair quality studies resulted in non-significant risk reduction. Reductions in AAD and CDAD were found regardless of whether a primarily *Lactobacillus*-based probiotic or an *S. boulardii*-based formulation was used (Pattani *et al.* 2013).

It has been suggested that follow-up in many studies is not sufficient to cover the whole period of risk for AAD/CDAD (Allen *et al.* 2013). One large multicentre, randomised, double-blind, placebo-controlled trial (not included in above meta-analyses) in the UK found no evidence that probiotic administration in older patients (≥65 years) was effective in preventing AAD. In this study, 2981 patients were recruited and were given either a probiotic combination of two strains of *Lactobacillus acidophilus* [CUL60/National Collection of Industrial, Food and Marine Bacteria (NCIMB) 30157 and CUL21/NCIMB 30156] and two strains of *Bifidobacteria* (*Bifidobacterium bifidum* CUL20/NCIMB 30153 and *Bifidobacterium lactis* CUL34/NCIMB 30172), or placebo for 21 days (around half the participants took the probiotic or placebo for the full 21 days). The main outcome measure was occurrence of AAD within 8 weeks and CDAD within 12 weeks of recruitment. The study findings showed that frequency of AAD (including CDAD) was similar in the probiotic (10.8%) and placebo arms (10.4%) resulting in a similar risk for developing AAD. CDAD was an uncommon cause of AAD and occurred in 0.8% of participants in the probiotic and 1.2% in the placebo arm, resulting in a non-significant risk reduction of 29%. The study authors concluded that on balance, the administration of the probiotic combination used in this study seems unlikely to benefit older patients exposed to antibiotics (Allen *et al.* 2013).

- Systematic reviews and meta-analyses have found that probiotics can lower the risk of CDAD by more than 60% in children and adults (outpatients), and in adult inpatients. However, one recent large multicentre trial from the UK did not find a beneficial effect of a combination of two *Bifidobacteria* and two *Lactobacilli* strains in hospitalised patients (although the occurrence of CDAD was generally very low).

ACUTE DIARRHOEA

Acute diarrhoea can have several causes, including bacterial or viral infections, and is a common cause of childhood morbidity and hospital admission. In developing countries acute diarrhoea is the leading cause of morbidity and mortality in children and an important cause of malnutrition (WHO 2004).

A Cochrane review published in 2010 sought to assess the evidence on the effectiveness of probiotics in acute infectious diarrhoea (ongoing for <14 days), including randomised and quasi-randomised controlled trials in both children and adults (Allen *et al.* 2010). Overall, 63 studies were included, testing many different probiotics. Three probiotic strains were used in several studies: *L. rhamnosus* GG (13 studies), *S. boulardii* (10 studies) and *Enterococcus* lactic acid bacteria SF68 (5 studies), allowing further sub-analysis. The review found that probiotics generally reduced the duration of diarrhoea, with differences varying widely between studies (-79.2 to 7.0 hours). Despite the high level of the quantitative heterogeneity, the authors concluded that the pattern was striking. Meta-analysis showed that probiotics significantly reduced the mean duration of diarrhoea by almost 25 hours. The risk of diarrhoea lasting ≥4 days was 59% lower in those taking probiotics compared to control, and stool frequency on day 2 of intervention was significantly lower (mean difference 0.8). *L. rhamnosus* GG significantly reduced the mean duration of diarrhoea by almost 27 hours and reduced the risk of

diarrhoea lasting ≥4 days by 41%, while *Enterococcus* lactic acid bacteria SF68 reduced the risk of diarrhoea lasting ≥4 days by 79% and *S. boulardii* by 63%. The review authors concluded that, used alongside rehydration therapy, probiotics appear to be safe and have clear beneficial effects in shortening the duration and reducing stool frequency in acute infectious diarrhoea, but that more research is needed to guide the use of particular probiotic regimens in specific patient groups (Allen *et al.* 2010).

A systematic review of RCTs investigated the effectiveness of *S. boulardii* in acute diarrhoea in both ambulatory and hospitalised patients. Nineteen relevant studies were identified (17 in children), and 13 were included in meta-analyses. Treatment with *S. boulardii* compared to placebo reduced the duration of diarrhoea by an average of 1 day and significantly reduced the risk of diarrhoea at the third day of illness by 48% (Dinleyici *et al.* 2012).

A meta-analysis investigating the effect of *L. rhamnosus* GG supplementation for the prevention of healthcare-associated diarrhoea in children found that the probiotic significantly reduced the risk of diarrhoea by 63%, although this was based on findings from only 2 RCTs. Pooling data from 3 RCTs resulted in a significantly reduced risk of symptomatic rotavirus gastroenteritis in the probiotic group compared with placebo (by 51%), while no significant difference in the incidence of asymptomatic rotavirus infection was found (2 RCTs). The authors concluded that in hospitalised children, the administration of *L. rhamnosus GG* has the potential to reduce the overall incidence of healthcare-associated diarrhoea, although they emphasise that studies did not include high-risk patients (e.g. in intensive care) (Szajewska *et al.* 2011).

Another Cochrane review investigated the effect of probiotics in persistent diarrhoea (lasting more than 14 days) in children. Only a limited number of studies are available with four trials with a total of 464 participants being eligible for inclusion. Meta-analysis of findings from two RCTs showed that probiotics significantly reduced the duration of persistent diarrhoea by an average of 4 days, while two of the RCTs found reduced stool frequency (Aponte *et al.* 2013).

- Probiotics are effective in treating acute diarrhoea and shorten its duration by an average of one full day. The most studied probiotics are *L. rhamnosus* GG, *S. boulardii* and *Enterococcus* lactic acid bacteria SF68, which all were effective.
- The evidence on the use of probiotics in persistent diarrhoea (lasting more than 14 days) is limited but suggests that probiotics may be effective in shortening duration of diarrhoea.

IMMUNE SYSTEM

The immune system is complex involving many different cell types distributed throughout the body and many different chemical mediators. It has been suggested that certain probiotics can regulate critical components of the immune system, such as lymphocytes, antibodies and natural killer cells (Sanders *et al.* 2013). A large body of evidence exists investigating the effect of probiotics on single immune parameters; however, summarising these studies is out of scope of this paper. Instead, this section will focus on studies investigating the effect of probiotic use on risk of respiratory tract infections (including the common cold) as the main outcome measure.

A recently published Cochrane review investigated the effect of probiotics for preventing acute upper respiratory tract infections (URTI). The review included RCTs that compared probiotics (mostly *Lactobacillus* and *Bifidobacterium* strains) with either placebo or no treatment. Thirteen relevant trials were identified, although data for meta-analyses was only available from 12 studies involving 3720 participants including children, adults and older people. Overall, use of probiotics reduced the number

of participants experiencing episodes of URTI by about 47% and the duration of an episode of acute URTI by almost 2 days; both findings were statistically significant. In addition, the authors found that probiotics may slightly reduce the use of antibiotics and cold-related school absence. However, the review authors suggested that the results must be interpreted with caution due to the overall low quality and risk of bias of the included studies. Overall, the authors judged the evidence as low or very low (Hao *et al.* 2015).

Another systematic review and meta-analysis investigated the effectiveness of probiotics (mostly Lactobacilli and Bifidobacteria) on the duration of illness in children and adults who developed common acute infectious respiratory conditions, affecting both the upper and lower respiratory tract. Pooling of data from nine trials resulted in a shorter duration of illness episodes (by about 0.8 days) in those taking probiotics, although there was significant heterogeneity between studies. Six studies considered to have a low risk of bias yielded similar results. There was also a statistically significant beneficial effect on number of days of illness and absenteeism, although the effect sizes were medium to small (King *et al.* 2014). An important limitation of this review is that some included trials investigated 'common infectious diseases', which would have included patients developing GI infections, which may have biased the results. However, overall the authors suggest that most included RCTs are of good quality (King *et al.* 2014). A meta-analysis of RCTs investigating the effect of probiotics on prevention of common cold found no evidence for a beneficial effect (Kang *et al.* 2013).

- Evidence suggests that probiotics have the potential to lower the risk of URTI and also shorten duration of episodes. However, the quality of the available evidence may be low.

ALLERGY

Allergy is an abnormal response of the immune system to contact with a foreign substance (an allergen) (British Nutrition Foundation 2002). It has been suggested that probiotics may be able to lower the risk of developing allergies for several reasons. A number of studies have suggested that the gut flora has an impact on the risk of developing allergies, and that the gut flora of children suffering from allergies differs from that of healthy children (Weichselbaum 2009). The 'hygiene hypothesis' suggests that a lack of exposure to microbes in early life can affect development of the immune system and increase susceptibility to certain disorders, such as allergies (Bach 2002). The most studied form of allergic disease in relation to the use of probiotics include atopic dermatitis (or eczema) and asthma, which will be discussed in more detail.

A systematic review and meta-analysis of randomised placebo-controlled trials investigated the effect of probiotics supplementation during pregnancy or infancy on risk of atopic dermatitis. Pooling data from 13 trials resulted in a significantly reduced risk (by 21%) of atopic dermatitis when probiotics were administered during pregnancy and/or during infancy. A similar risk reduction (20%) was observed for immunoglobulin E (IgE)-associated atopic dermatitis. The most commonly studied probiotics were *L. rhamnosus* GG and probiotic combinations. When only including studies using *L. rhamnosus* GG risk of atopic dermatitis was significantly reduced by 26%. Sub-group analysis found that risk reduction was only significant if probiotics were taken pre- and post-delivery, but not when taken post-delivery only. Similarly, the effect was only significant when either the mother or the mother and child received the probiotic, but not when the child only was given probiotics (Pelucchi *et al.* 2012). Another systematic review and meta-analysis looked at atopic sensitisation in general and found that administration of probiotics during pregnancy and infancy was associated with a statistically significant 12% reduction in atopic sensitisation, while administration postnatally alone

did not reduce the risk. The researchers also found that probiotics were effective in reducing total IgE in children with atopy (Elazab *et al.* 2013).

A recently published meta-analysis of RCTs investigated whether probiotics are effective in treating atopic dermatitis. Pooling data from 25 trials including 1599 subjects resulted in a significant effect of probiotics treatment on symptoms of atopic dermatitis, although significant heterogeneity among studies was observed. Further analysis found that probiotics were effective in children (1-18 years) and adults (>18 years), but not in infants (<1 year). Probiotic mixtures and *Lactobacillus* strains were found effective, while treatment with *Bifidobacterium* strains showed negative effects. Those who had moderate to severe AD benefited most from probiotic administration while there was no treatment effect in those with mild disease. Despite the statistically significant beneficial effect of probiotic treatment, the study authors questioned the clinical significance of the effect size (mean reduction of 4.5 points on a scale of 0-103) (Kim *et al.* 2014).

Evidence to date does not support a beneficial effect of probiotic supplementation during pregnancy and/or infancy on the prevention of asthma and wheeze (Azad *et al.* 2013; Elazab *et al.* 2013).

Other forms/manifestations of allergy such as allergic rhinitis and general risk of allergic sensitisation are less well studies and/or evidence is conflicting (Weichselbaum 2009). Food allergies are closely linked to atopic dermatitis, suggesting that probiotics may have a role to play in prevention and treatment of food allergy. This has sparked research interest in this area, but to date there is no evidence to support a role of probiotics in food allergy prevention and management (Nermes *et al.* 2013).

- Evidence suggests that probiotic administration during pregnancy, and during pregnancy and infancy reduces the risk of atopic dermatitis and atopic sensitisation, while supplementation during infancy only does not seem to be effective. The most commonly studied strain is *L. rhamnosus* GG, which proved effective in lowering the risk of atopic dermatitis, as were combinations of probiotic strains.
- There is evidence to suggest that *Lactobacillus* strains, but not *Bifidobacteria*, may be effective in treating atopic disease in children and adults (but not in infants <1 year), although the observed improvements are small and may not be clinically significant.
- Evidence to date does not support a beneficial effect of probiotic supplementation during pregnancy and/or infancy on the prevention of asthma and wheeze.

PROBIOTICS IN INFANT FEEDING

Probiotics, as well as prebiotics, are increasingly used in infant formulae in an attempt to promote a gut microflora resembling more closely that of breastfed infants (breastmilk naturally contains prebiotics and live bacteria). In 2011, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition published a systematic review and comment on the supplementation of infant formula with probiotics and prebiotics. While the committee concluded that administration of probiotics through formula to healthy infants does not raise safety concerns with regard to growth and adverse effects, it was also concluded that there was insufficient data to recommend the routine use of probiotic-supplemented formulae. The committee suggested that the evidence was too limited for health outcomes investigated (including growth, GI infections, respiratory symptoms, colic and crying) to draw reliable conclusions, with research at the time having failed to find significant beneficial effects. The committee, however, also considered that the supplementation of formula with probiotics (and/or prebiotics) is an important field of research

and that there is a need for well-designed RCTs, with relevant inclusion/exclusion criteria and adequate sample sizes (ESPGHAN Committee on Nutrition 2011). A systematic review published a year later came to similar conclusions and supported the findings of ESPGHAN (Mugambi *et al.* 2012a). Mugambi and colleagues also systematically reviewed the evidence around probiotics use in preterm or low birth weight infants, and also came to the conclusion that there is not enough evidence to state that supplementation with probiotics results in improved growth and clinical outcomes in exclusively formula fed preterm infants, highlighting a lack of evidence in this space (Mugambi *et al.* 2012b).

A Cochrane review published in 2011 investigated the use of probiotics for the prevention of necrotising enterocolitis (NEC) and nosocomial sepsis in preterm infants, which are conditions associated with increased morbidity and mortality. The review included evidence from RCTs and quasi-RCTs. Based on 16 trials the authors concluded that enteral supplementation of probiotics significantly reduces the risk of NEC by 65% and mortality by 60%, while no effect on risk of nosocomial sepsis was found (AlFaleh *et al.* 2011). An updated analysis including 24 trials supported the earlier findings, although the risk reduction was somewhat smaller (57% for NEC and 35% for mortality) (AlFaleh & Anabrees 2014). These findings have been used by some expert committees to recommend the use of probiotics as a means to decrease the incidence of NEC (e.g. by the American Pediatric Surgical Association Outcomes and Clinical Trials Committee, Downard *et al.* 2012). However, a group of experts from nine European countries and Israel criticised the approach by AlFaleh *et al.* and other reviews that have come to similar conclusions, suggesting meta-analyses of data from included studies were inappropriate due to fundamental methodological differences between the study protocols (Mihatsch *et al.* 2012). The experts suggest that these meta-analyses have only hypothesis generating power since they are based on exploratory data analysis, and not on confirmatory trials. In their own systematic review published in 2012 they found that out of 16 included trials only two were of high quality, while the rest was of low quality and could not be used to draw conclusions on the effectiveness of probiotics in the prevention of NEC. The authors suggest that there is insufficient evidence to recommend routine probiotics for the prevention of NEC and sepsis, but that there is encouraging data from lower quality studies which justifies the further investigation regarding the efficacy and safety of specific probiotics in circumstances of high local incidence of severe NEC (Mihatsch *et al.* 2012).

The evidence on the use of probiotics during infancy for the prevention of atopic dermatitis and asthma is discussed above. In summary, evidence to date suggests that administration of probiotics during infancy reduces the risk of atopic dermatitis only if the probiotic was also administered during pregnancy, while the use of probiotics during infancy alone has no significant effect. No effect on the risk of asthma and wheeze was found with probiotic administration during pregnancy and/or infancy. For more details see 'Allergy' section.

- Evidence on the use of probiotics in infant formula is still emerging and looks promising for certain aspects, in particular the prevention of NEC.
- Evidence suggests the use of probiotics in infant formula is safe (also see 'Safety of probiotics').

EMERGING EVIDENCE

OBESITY / BODY WEIGHT

Obesity is a major health issue throughout the world and research to date has been unable to conclusively ascertain the determinants underlying the epidemic of obesity. Beside factors such as excessive food intake and lack of physical activity, more recently the gut microbiota has attracted

increasing attention in obesity research in terms of nutrient processing, extraction and utilisation. Dietary intake not only affects energy balance but also constantly regulates and modifies the microbiota composition, which influences nutrient accessibility for the host body, and thereby potentially boosts weight gain. There is evidence of a gut microbiota that facilitates the extraction of energy from the ingested diet and its storage in the host adipose tissue (Luoto *et al.* 2013). It has been suggested that dysbiosis, which is the perturbation of the gut microbiota composition, could promote intestinal monosaccharide absorption and energy extraction from non-digestible food components (mainly carbohydrates) via short-chain fatty acid production and hepatic *de novo* lipogenesis. Other possible mechanisms have also been suggested. It has further been suggested that modification of the gut microbiota by specific dietary or pharmacological interventions may favourably affect host metabolism and may help with weight control. The evidence of a direct impact of gut microbiota modulation on weight development is, however, so far scant (Luoto *et al.* 2013). One study found that administering *L. gasseri* SBT2055 to overweight subjects significantly diminished abdominal adiposity and body weight (Kadooka *et al.* 2010). However, more studies are needed to establish whether probiotics are a useful means for weight management.

Pregnancy and early infancy have been identified as critical stages and targets for interventions aiming to reduce the risk of overweight development in future generations. Initial microbial colonisation of the GI tract may be an instrumental contributor to the infant's weight development, and it has been suggested that newborns constitute one of the populations most likely to benefit from the use of probiotics (Luoto *et al.* 2013). In one study, infants whose mothers received a probiotic combination of *L. rhamnosus* GG and *B. lactis* during pregnancy showed an increase in bifidobacterial diversity during the first year of life compared to the placebo group. These differences were found to predict overweight in children early in life, those becoming overweight by 7 years of age having had lower levels of *Bifidobacteria* and higher levels of *Staphylococcus aureus* at 6 and 12 months of age compared to those remaining normal weight (Kalliomäki *et al.* 2008). The probiotic intervention moderated excessive weight gain especially among children who subsequently became overweight during the first years of life, the impact being most pronounced at the age of 4 years (Luoto *et al.* 2010).

This area of research is still fairly new and more research is needed.

BONE HEALTH

There is emerging evidence, mostly from animal studies, that probiotics may positively affect bone metabolism and bone density (Parvaneh *et al.* 2014, Scholz-Ahrens *et al.* 2007). To date only one human study assessed the effect of probiotics on bone health. In this study 20 postmenopausal women consumed *L. helveticus* fermented milk or control milk. Consumption of the probiotic led to a reduction of parathyroid hormone, which consequently reduced bone resorption (Narva *et al.* 2004). Possible mechanisms by which probiotics may positively affect bone health include increased mineral solubility in the gut due to increased production of short-chain fatty acids; production of phytase enzyme by bacteria in the gut, thereby increasing bioavailability of minerals from plant foods; increased synthesis of vitamins involved in bone health, such as vitamin D, C and K; reduced blood parathyroid hormone and increased blood calcium levels (Parvaneh *et al.* 2014, Scholz-Ahrens *et al.* 2007). However, overall the evidence is still limited and more studies are needed to confirm a beneficial effect of probiotics on bone health.

SAFETY OF PROBIOTICS

The safety of probiotics was systematically investigated in a report prepared for the U.S. Department of Health and Human Services. One of the main findings of this investigation was that in general, adverse events are poorly documented. While many studies do report on the safety of probiotics, most did not state what adverse events were monitored and did not systematically address the safety of the probiotic products. Where adverse events were reported, none of the studies reported an infection (such as fungemia, bacteremia, sepsis or other infections) caused by administered probiotic organisms. Across all included studies, the most commonly reported adverse events were gastrointestinal in nature. However, there was no indication that participants using probiotic organisms experienced more GI or other adverse events compared to control group participants. The report concludes that the current available evidence does not suggest a widespread risk of adverse events associated with probiotics, but that future studies that explicitly monitor for the issues of concern are needed to quantify the actual risk of specific adverse events (AHRQ 2011).

A systematic review investigating the safety of probiotics and synbiotics in infants under two years of age also highlighted a lack of precise reporting and classification of adverse events in most studies. Analysis of 57 clinical trials indicated that probiotic administration to infants between 0 and 24 months was safe. Most adverse events and serious adverse events were considered unrelated to the study product, and there were no major safety concerns. However, the authors suggest that inconsistent, imprecise and potentially incomplete reporting as well as the variation in probiotic strains, dosages, administration regimes, study populations and reported outcomes, greatly limit the generalisability of conclusions. They recommend that each new probiotic strain needs to undergo thorough examination to ensure that it is safe to use in infants (van den Nieuwboer et al. 2014).

DISCUSSION (INCLUDING REGULATORY ISSUES)

There is good evidence to support the use of probiotics for treatment or prevention of certain health issues, including antibiotic-associated diarrhoea, acute diarrhoea, constipation, eczema and ulcerative colitis, while for other health issues the evidence is less clear for other areas such as irritable bowel syndrome and risk of common cold. Conflicting results may be due to different study designs used, and in particular due to different probiotic strains tested. Many of the health effects of probiotics are strain specific, meaning that while one specific strain may be effective in treating or preventing a certain health issue, another one may not be. This can lead to conflicting findings, and when pooling such data the effectiveness of certain strains may be underestimated. However, combining data in meta-analyses is, despite its limitations, useful as many studies are underpowered and may by themselves not find any significant results. The results of meta-analyses and reviews merging results of studies using different strains can give an overview of the health potential or probiotics in general, but it is hard to draw conclusions from such studies about which strains are effective and which are not. Some of the reviews included in this paper have highlighted that some studies are of rather low quality. More high-quality studies are needed for some of the areas to find out whether probiotics are effective or not.

Although the evidence shows a clear benefit of probiotics in the treatment or prevention of certain health issues, the European Food Safety Authority (EFSA), which provides scientific advice to the European Commission, has so far rejected any health claims for use on food products that suggest that healthy individuals benefit from taking probiotics. This has led to much confusion regarding the benefits of probiotics among consumers, while researchers in the field of probiotics have perceived EFSA's decision as a backlash to their findings to date. Although there seems to be a discrepancy between EFSA's rejections of health claims relating to probiotics and evidence showing a clear benefit for some conditions, the critical point is that for health claims aimed at the general population to be permitted, evidence needs to show a clear effect in *healthy* people. However, most studies have been carried out in people with an existing condition or at high risk of developing a certain health issue. While these studies provide support for the use of probiotics in people with existing or at high risk of health conditions, they do not provide proof of benefit for healthy people. Finding health benefits in already healthy people is more challenging that finding benefits in people with health issues. More research is underway, using newer technologies and specific biomarkers that may help understand whether or how healthy individuals may benefit from the use of probiotics.

Overall, evidence suggests that there is a clear benefit of taking probiotics in order to prevent or treat certain health issues. A diversity of strains have been studied, making it difficult to identify which specific strains are effective, although for certain areas such as AAD a variety of strains seem to be effective. More research is needed to identify whether and how healthy consumers may benefit from taking probiotics.

RECOMMENDATIONS AND KEY MESSAGES

- Probiotics seem more effective than placebo in reducing symptoms in IBS sufferers, in both adults and children. Combination probiotics seem effective, while no significant effect for the use of single strains (or genera) was found. However, this could be due to the small number of studies using single strains with insufficient power to detect any meaningful effect.

- Overall, the evidence to date suggests that IBS sufferers may benefit from using probiotics.

- Probiotics seem effective in treatment of constipation in adults by increasing stool frequency and consistency. Further investigation found that probiotics of the species *B. lactis* were effective, while *L. casei* Shirota failed to improve symptoms of constipation. Probiotics have to date failed to convey a benefit in constipated children.

- The use of probiotics in treatment and management of UC is promising. *E. coli* Nissle 1917 was found to be as effective as the gold standard treatment in maintaining remission, and the probiotic combination VSL#3 when given with standard treatment was significantly superior to placebo in improving the UC disease activity. For other strains or combinations of strains the evidence to date is limited.

- The probiotic combination VSL#3 was effective in reducing relapse rate in patients with chronic/recurring pouchitis in remission, was effective in reducing the risk of developing pouchitis following surgery for UC, and may be effective in treating mild active pouchitis. *L. rhamnosus* GG was not found to be effective in treating active pouchitis.

- Evidence to date does not suggest that probiotics are effective in the treatment and management of CD.

- Probiotics are effective in reducing the risk of AAD in both children and adults by around 42-48%, but may not be effective in older adults. There was no evidence that effects differed between different strains, although strains were often not well documented.

- Systematic reviews and meta-analyses have found that probiotics can lower the risk of CDAD by more than 60% in children and adults (outpatients), and in adult inpatients. However, one recent large multicentre trial from the UK did not find a beneficial effect of a combination of two *Bifidobacteria* and two *Lactobacilli* strains in hospitalised patients (although the occurrence of CDAD was generally very low).

- Probiotics are effective in treating acute diarrhoea and shorten its duration by an average of one full day. The most studied probiotics are *L. rhamnosus* GG, *S. boulardii* and *Enterococcus* lactic acid bacteria SF68, which all were effective.

- The evidence on the use of probiotics in persistent diarrhoea (lasting more than 14 days) is limited but suggests that probiotics may be effective in shortening duration of diarrhoea.

- Evidence suggests that probiotics have the potential to lower the risk of URTI and also shorten duration of episodes. However, the quality of the available evidence may be low.

- Evidence suggests that probiotic administration during pregnancy, and during pregnancy and infancy reduces the risk of atopic dermatitis and atopic sensitisation, while supplementation during infancy only does not seem to be effective. The most commonly studied strain is *L. rhamnosus* GG, which proved effective in lowering the risk of atopic dermatitis, as were combinations of probiotic strains.

- There is some evidence to suggest that *Lactobacillus* strains, but not *Bifidobacteria*, may be effective in treating atopic disease in children and adults (but not in infants <1 year), although the observed improvements are small and may not be clinically significant.

- Evidence to date does not support a beneficial effect of probiotic supplementation during pregnancy and/or infancy on the prevention of asthma and wheeze.

- Evidence on the use of probiotics in infant formula is still emerging and looks promising for certain aspects, in particular the prevention of NEC. Evidence suggests the use of probiotics in infant formula is safe.

- There is emerging evidence for a role of probiotics in treatment of obesity and for bone health, but more evidence is needed before any conclusions can be drawn.

- Use of probiotics is generally considered safe for the general population, although a more systematic approach to monitor adverse events is warranted.

- Overall, probiotics can be used for the prevention and/or treatment of a variety of health issues. A diversity of strains have been studied, making it difficult to identify which specific strains are effective, although for certain areas such as AAD a variety of strains seem to be effective

- Research is underway to understand whether and how healthy consumers can benefit from taking probiotics.

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