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REVIEW ARTICLE

Probiotics: an update☆

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Abstract
Objective: Triggered by the growing knowledge on the link between the intestinal microbiome and human health, the interest in probiotics is ever increasing. The authors aimed to review the recent literature on probiotics, from definitions to clinical benefits, with emphasis on children.
Sources: Relevant literature from searches of PubMed, CINAHL, and recent consensus statements were reviewed.
Summary of the findings: While a balanced microbiome is related to health, an imbalanced microbiome or dysbiosis is related to many health problems both within the gastro-intestinal tract, such as diarrhea and inflammatory bowel disease, and outside the gastro-intestinal tract such as obesity and allergy. In this context, a strict regulation of probiotics with health claims is urgent, because the vast majority of these products are commercialized as food (supplements), claiming health benefits that are often not substantiated with clinically relevant evidence. The major indications of probiotics are in the area of the prevention and treatment of gastro-intestinal related disorders, but more data has become available on extra-intestinal indications. At least two published randomized controlled trials with the commercialized probiotic product in the claimed indication are a minimal condition before a claim can be sustained. Today, *Lactobacillus rhamnosus* GG and *Saccharomyces boulardii* are the best-studied strains. Although adverse effects have sporadically been reported, these probiotics can be considered as safe.
Conclusions: Although regulation is improving, more stringent definitions are still required. Evidence of clinical benefit is accumulating, although still missing in many areas. Misuse and use of products that have not been validated constitute potential drawbacks.

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PALAVRAS-CHAVE
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Probióticos: informações atualizadas

Resumo

Objetivo: Motivado pelo conhecimento cada vez maior da associação entre o microbioma intestinal e a saúde humana, o interesse nos probióticos vem crescendo cada vez mais. Os autores visaram analisar a última literatura a respeito dos probióticos, de definições a benefícios clínicos com ênfase nas crianças.

Fontes dos dados: Foi analisada a literatura relevante de pesquisas do PubMed, do CINAHL e dos últimos consensos.

Síntese dos dados: Apesar de um equilíbrio no microbioma estar relacionado à saúde, um desequilíbrio no microbioma ou disbiose está relacionado a vários problemas de saúde no trato gastrointestinal, como diarreia e doença inflamatória intestinal, e fora do trato gastrointestinal, como obesidade e alergia. Nesse contexto, a regulamentação rigorosa dos probióticos a alegações de saúde é urgente, pois a grande maioria desses produtos é comercializada como alimentação (suplementos), alegando benefícios à saúde que frequentemente não são comprovados com evidências clinicamente relevantes. As principais indicações de probióticos são feitas na área da prevenção e tratamento de doenças gastrointestinais, porém mais dados têm sido disponibilizados a respeito de indicações extraintestinais. Pelo menos dois ensaios clínicos controlados e randomizados publicados com o probiótico comercializado na indicação declarada são a condição mínima antes de uma afirmação poder ser mantida. Atualmente, o *Lactobacillus rhamnosus* GG e *Saccharomyces boulardii* são as melhores cepas estudadas. Apesar de efeitos adversos terem sido esporadicamente relatados, os probióticos podem ser considerados seguros.

Conclusões: Apesar de a regulamentação estar aumentando, ainda são necessárias definições mais rigorosas. As evidências de benefícios clínicos estão aumentando, apesar de ainda ausentes em várias áreas. O uso inadequado e a utilização de produtos não validados constituem possíveis desvantagens.

Introduction

The joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) Expert Consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria defined probiotics as: ''live microorganisms that, when administered in adequate amounts, confer a health benefit on the host''.[1] In 2002, a joint FAO/WHO Working Group[2] released guidelines for the evaluation of probiotics in food. The minimum requirements needed for probiotic status include:

- assessment of strain identity (genus, species, strain level);
- *in vitro* tests to screen potential probiotics: e.g. resistance to gastric acidity, bile acid, and digestive enzymes, antimicrobial activity against potentially pathogenic bacteria;
- safety assessment: requirements for proof that a probiotic strain is safe and without contamination in its delivery form;
- *in vivo* studies for substantiation of the health effects in the target host.

Following the FAO/WHO definition, the International Life Science Institute (ILSI)[3] and the European Food and Feed Cultures Association (EFFCA)[4] have released similar definitions for a probiotic: ''a live microbial food ingredient that, when consumed in adequate amounts, confers health benefits on the consumers'' and ''live microorganisms that, when ingested or locally applied in sufficient numbers, provide the consumer with one or more proven health benefits''. These definitions *de facto* imply that probiotic ingestion provides benefits for host health.

The science related to probiotics is recent, and is thus in constant evolution. Probiotics used in food, supplied as dietary supplement, or as active components of a registered medication, should not only be capable of surviving passage through the digestive tract by exhibiting acid and bile survival, but should also have the capability to proliferate in the gut. Probiotics must be able to exert their benefits on the host through growth and/or activity in the human body. Topical or local application of probiotics is also proposed in view of the recent evolution of scientific data. Therefore, the ability to remain viable and effective at the target site should be studied and confirmed for each strain, or even better, for each commercialized product. Clinical studies should be performed with the commercialized product and not with the isolated strain. However, lack of protection contributes to the fact that some companies refuse to deliver information on the specific strains in their product.[5] Recent literature has demonstrated that one of the mechanisms of action of probiotics involves stimulation of the immune system. It is questionable whether the probiotics need to be ''alive'' to induce immune-modulation. Therefore, the definition may have to be revised in the future.

According to the European Union (EU), health claims should only be authorized for use in the EU after a scientific assessment of the highest possible standard has been carried out by the Panel on Dietetic Products, Nutrition, and Allergies (NDA) of the European Food Safety Authority (EFSA) [Regulation (EC) N 1924/2006].[6] The key questions that were addressed by the EFSA NDA panel are:

- Is the food/constituent sufficiently defined and characterized?
- Is the claimed effect sufficiently defined, and is it a beneficial physiological effect?
- Have pertinent human studies been presented to substantiate the claim?

The EFSA recommendations are an important step forward in trying to bring claims for probiotic food supplements and medication closer together. However, companies discovered side-ways to avoid EFSA restrictions. Some of the food supplements are in the process of registration as ''medical device'', for which claims can be made without providing hard scientific evidence. Moreover, production requirements on quality control and safety still differ substantially between food supplements and medication, putting medication in a disadvantageous situation.

Official controls by national authorities are performed to ensure compliance with food law. Apart from the risk of using unauthorized strains, product mislabeling is a known problem, partly due to the use of phenotyping or genotyping methods that lack discriminative power.[7] In addition to official controls, private controls by food producing companies are important in the context of protection of patented strains and industrial property rights.

In their 'Guidelines for the Evaluation of Probiotics in Food', the FAO/WHO Working Group[2] recommends that the following information should be described on the label of probiotic products:

- Genus, species, and strain designation. Strain designation should not mislead consumers regarding the functionality of the strain;
- Minimum viable numbers of each probiotic strain at the end of the shelf-life;
- Suggested serving size, which must deliver the effective dose of probiotics related to the health claim;
- Health claim(s);
- Proper storage conditions;
- Corporate contact details for consumer information.

In most countries, only general health claims are currently allowed on foods containing probiotics. The FAO/WHO Working Group[2] recommended that specific health claims on foods should be allowed relating to the use of probiotics, whenever sufficient scientific evidence is available. Such specific health claims should be permitted on the label and in promotional material. For example, a specific claim stating that a probiotic 'reduces the incidence and severity of rotavirus diarrhea in infants' would be more informative to the consumer than a general claim that states 'improves gut health'. The recommendation is that the product manufacturer should be responsible for conducting an independent third party review by scientific experts in order to establish the truthfulness of the health claims.

In line with the suggestions of the FAO/WHO Working Group,[2] on December 20, 2006, the European Parliament and the Council published a new regulation (No. 1924/2006) regarding ''Nutrition and Health Claims Made on Foods''.[6] This regulation applies to all nutritional and health claims relating to all types of food intended for final consumers; thus including probiotic products brought to the market with a health claim. The regulation aims to consolidate the nutrition and health claims at European level in order to better protect consumers, including commercial communications (labeling, presentation, and promotional campaigns), as well as trademarks and other brand names that could be construed as nutrition or health claims.

Functional effects of probiotics

The definition ''probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host'' only generalizes the probiotic functionality as conferring a health benefit to the host. Hence, this definition entails that there must be a measurable physiological benefit to the host who uses the probiotic product. In addition, it is not specified that the probiotic strain must be provided through oral delivery, nor are there specific requirements regarding the mode of action. The latter also implies that survival of the probiotic microorganisms throughout the gastrointestinal (GI) tract is not a prerequisite for recognition of probiotic effects. For example, the delivery of lactase through administration of live *Streptococcus thermophilus* to the small intestine can be considered as a probiotic activity, although the bacterial strain itself does not survive the digestive tract.[8]

When considering probiotic functionality, the abovementioned definition of probiotics is to be interpreted in a very broad way. This broad functionality definition complicates the process of functional characterization of probiotics. The use of probiotics may target several body sites (mouth, GI tract, respiratory tract, urinary tract, skin, vagina, etc), and its application can also target specific human subpopulations: healthy individuals, children, elderly, ill subjects, and immunecompromised and genetically predisposed individuals, among others. There is an extremely diverse range of potential biological effects, and new functional activities are constantly being explored. While some models are perfectly suited for studying the colonization potency of probiotics, other models need to be applied for assessing their immune-modulating potential, their resilience against pathogen invasion of the GI tract, or their anti-inflammatory properties.

Functional characterization of probiotics

Target sites

Probiotic products have been developed to improve physiological conditions at different body sites. While the GI tract

is the most important target for the majority of probiotic applications, other body sites, such as the mouth, the urogenital tract, and the skin, are also considered. Probiotics may play an important in oral medicine and dentistry.[9,10]

Probiotics are also considered for controlling and preventing infections of the reproductive and urinary tract.[11–14] Regarding skin applications, probiotics may be consumed orally to induce an immune response that has systemic effects, e.g., for controlling skin inflammation[15] and dermatological diseases in general.[16] Probiotics have also been used as protection against respiratory tract infections. *Lactobacillus (L.) rhamnosus GG* prevents respiratory tract infections in addition to the conventional protection against GI infections.[17] There is a plethora of probiotic strains and applications available with the GI tract as target site. Such applications aim at several health benefits, such as decreasing pathogen colonization, improving vitamin synthesis, optimizing intestinal transit, alleviating lactose intolerance, reducing bloating, and promoting immunemodulatory effects, among others.

Delivery mode

In order to provide health benefits, probiotic strains often require a specific matrix to guarantee optimal strain survival along the GI tract. For instance, probiotics have recently been formulated in a chocolate matrix, which resulted in a more optimal survival of the probiotic strains in comparison with conventional probiotic formulation methods.[18] Other methods include the introduction of probiotics in more conventional products such as milk,[19] kefir,[20] and yoghurts, or in more specific matrices such as cereals, cheese, sausages, and cookies. Obviously, many probiotics are introduced for commercial reasons, to obtain a better product placement or to integrate the probiotic market (common examples are fruit juices, ice creams, candies, granola bars, etc).

Besides the incorporation of probiotics in food products, probiotic strains are also provided as food supplement, often targeting specific health problems. Probiotic food supplements (e.g. *L. rhamnosus GG, L. reuteri*) and medication (e.g. *Saccharomyces boulardii*) have almost become standard in the treatment of pediatric gastroenteritis. There are many infant formulas with probiotics, both to prevent and alleviate diarrhea.

L. lactis strains that secrete IL-10 or immunomodulatory *Yersinia* LcrV protein were developed to treat colitis in mouse models.[21,22] Such approach is currently being considered for the treatment of oral mucositis (high incidence in head/neck cancer patients receiving radiotherapy) with a human trefoil factor 1-secreting *L. lactis*. A molecular basis of the therapeutic applications and the chemopreventive activities of certain probiotic metabolites, with emphasis on the interaction between these metabolites and the molecular signaling cascades, are considered to be epigenetic targets in preventing colon cancer.[23]

Finally, probiotic delivery not only pertains the food or pharmaceutical environment in which the probiotic is formulated. Specific ointments and nasal sprays have been developed;[24] currently, even the introduction of probiotics in mattresses and cleaning agents is gaining momentum for an optimized hygienic control. The latter illustrates the need to broaden the control on claims larger than food supplements and food. If the EU installs authorities, such as the EFSA, to control claims for foods and food supplements, health-claims for non-food related products should be equally controlled.

Strain survival

In many cases, health benefits are only obtained when a probiotic strain reaches the target site in a metabolically active state and in sufficient numbers. For oral delivery, probiotic microorganisms must survive the different physicochemical, enzymatic, and microbial stresses throughout the GI transit.

Firstly, microorganisms have to cross the acidic environment from the stomach. In addition, the absence or presence of a food matrix significantly determines the pH profile to which the probiotic strain is subjected. While the initial pH buffering effect from food may subject the probiotic strain to initially less stringent acidic conditions, a longer digestion time in the stomach under fed conditions may expose part of the dosed probiotic to acidic conditions for a longer time. Many probiotic microorganisms have been selected for their higher resilience in such conditions, and new methodologies are available to allow encapsulation of probiotic strains in that purpose.[25]

A second stress component is the presence of bile salts that elicit membrane-compromising properties towards microorganisms, due to their amphiphilic character. A particular functional characteristic of microorganisms is their ability to deal with bile salt stress via bile salt hydrolase. Bile salt hydrolase bacteria typically cleave the glycin or taurin moiety from conjugated bile salts, rendering the latter less bacteriostatic. This feature is of particular importance to optimize strain survival during intestinal transit and has been proposed as a mechanism to explain how probiotics could lower serum cholesterol levels.[26]

Another feature of probiotic strain survival is the ability to colonize the GI tract. This property can be divided into an ecological component and a mucosal component. Firstly, once a probiotic organism has survived gastric acid and duodenal bile salts and thus reaches the ileum and colon, it has the possibility to develop in a less stringent environment. Yet, it reaches an environment with a highly significant microbial background – the ileum and colon reaching bacterial concentrations of 10^7 and 10^{11} cells/mL chyme, respectively. Obviously, a probiotic strain can be considered as foreign to the residing endogenous microbiota, and unless specific nutrients are provided for the probiotic in the product formulation (e.g. synbiotic), the strain must compete with the residing microbial community for available substrates. In ecological terms, the dosed probiotic must occupy a functional niche in the gut microbial ecosystem. Secondly, an important property for probiotics, e.g. with respect to pathogen control, is its ability to adhere to and thrive in the mucus surface that covers the gut epithelium. Mucosal adhesion can rely on cell wall properties. The hydrophobic nature of microbial strains can be assessed with a straightforward BATH assay,[27] while a specific mucus adhesion can be measured using short term adhesion assays with

gut-derived mucins (mostly from animal origin).[27,28] However, the aspecific adhesion of gut microorganisms to gut mucins is only sufficient for microcolony formation, and does not guarantee a prolonged colonization of the mucus layer. It has been well described that specific microorganisms modulate their gene expression following their incorporation into the mucosal surface. This has not only been described for pathogens,[28,29] but also for probiotic microorganisms, such as *L. rhamnosus GG*, which may upregulate the formation of specific pili in the mucosal environment.[30]

Human target groups

Probiotic products have been developed for a wide variety of health claims. Probiotics can target both healthy and ill individuals. The expected effects can be of a preventive or curative nature. The goal can be to fight the cause of the disease or metabolic alterations, or to lessen the symptoms associated with the occurrence or progression of a disease or metabolic alteration.

With the aim of improving health in the human body, the intake of a probiotic strain by healthy subjects has primarily preventive objectives. Yet, it must be emphasized that the introduction of a foreign strain – even if it is a probiotic – must be approached with care and must be performed after a well-considered evaluation process. Particularly, the gut environment from sensitive human subpopulations, such as infants and toddlers, undergoes a high degree of development or transition. Many studies on probiotic applications reported a positive outcome in markers that can be of relevance for human health. Probiotic studies have shown beneficial effects in all age-related subgroups, such as mother-infant pairs, preterm infants, newborns, infants, older children, and elderly people.

For example, fermented milk drinks with *L. casei* strain Shirota positively stimulate the immune system in healthy human subjects.[31] With respect to different age groups, the effects from long term consumption of probiotic milk on infections was evaluated in children attending day care centers,[32] while *L. delbrueckii* subsp. *bulgaricus* OLL1073R-1 was given to elderly persons with the aim of reducing the risk of infection.[33]

In case the microbial community from a specific body region is disturbed, leading to the so-called dysbiosis, functional niches become available in the ecosystem. Examples of dysbiosis are the changes in microbial ecosystem in the mouth associated with dental caries, or dysbiosis associated with bacterial vaginosis. Twetman recently reviewed the effects from probiotics on oral health in children.[34] For example, long-term application of probiotic strains such as *L. rhamnosus GG* lowered the risk of dental caries in children;[35] the importance of probiotic supplementation during orthodontic therapy was also reported.[36] Similarly, microbial dysbiosis in the urogenital tract, particularly bacterial vaginosis, can also be treated with probiotics.[37] *L. rhamnosus* GG and specific *L. acidophilus* strains have been used to treat bacterial vaginosis.[38,39] Probiotics may also be applied orally to reduce health risks that originate from the gut environment. *Helicobacter pylori*-colonized subjects have been treated with *L. casei* milk drinks[40] and *L. gasseri* OLL2716 (LG21),[41] while specific *Bifidobacterium* strains display anti-*Helicobacter* effects through the production of antimicrobial peptides.[42] However, there is proof that dysbiosis occurs in Crohn's disease (either as cause or consequence), but probiotic supplementation has always failed to prevent relapse, except in pouchitis. In addition, there is specific attention for developing probiotic concepts for children under modified risks. Preterm infants display an increased risk for developing necrotizing enterocolitis, which is decreased by the application of oral probiotics.[43]

Basis of the biological effect of probiotics

The health benefits from probiotic products and applications are extremely diverse and are continuously expanded with new insights and scientific developments.

Microbiological functionality

The ultimate goals of microbiological interventions through probiotics may be to stabilize or improve microbial homeostasis in a body environment and to lower pathogen invasion and colonization. The resilience of a microbial community against invasion by exogenous strains largely depends on the availability of non-occupied functional niches. If not all functional niches are occupied by the endogenous microbial community, there is an increased risk for pathogen invasion in the ecosystem, colonization, and subsequent infection.

Probiotic microorganisms can be used to improve or restore microbial homeostasis in two scenarios. Firstly, they may occupy functional niches that are left open by the endogenous community, thereby preventing (opportunistic) pathogens from occupying that niche. Such process is often referred to as competitive exclusion, and primarily targets the competition for nutrients, physical sites (e.g. mucus adhesion) or receptors. The second scenario is more of an antagonistic nature as probiotics may actively lower (opportunistic) pathogen invasion or development into the ecosystem. Such approach primarily targets: i) the production of short chain fatty acids and other organic acids (e.g. lactic acid) by probiotics, thereby lowering the pH and increasing the bacteriostatic effect of organic acids towards pathogens; ii) the production of bacteriocins, which are small microbial peptides with bacteriostatic or bactericidal activity; and iii) the production of reactive oxygen species, such as hydrogen peroxide, that are highly reactive and increase oxidative stress for pathogens in micro-environments.

Nutritional functionality

Specific microbial groups produce vitamins and may thereby contribute to vitamin availability to the human host. Apart from vitamin K,[44] vitamin B12,[45] and pyridoxine,[46] other vitamins, such as biotin, folate, nicotinic acid, and thiamine, can be produced by gut microorganisms. This type of activities may affect host health and may therefore be considered as potential probiotic effects.

Lactase deficiency causes lactose intolerance, which results in abdominal cramping, nausea, and bloating. Probiotic strains that are lactase-positive have been successfully applied to relieve discomfort from lactose intolerance.[47]

Other nutritional functionalities may include the production of health-promoting compounds. The metabolic potency of gut microorganisms is enormous and may rival or even exceed that of the liver.[48] The gut environment harbors several small chemical factories that produce numerous chemical components with putative health-modulating effects.[49] Isolated strains that produce health-promoting products may also be considered as having probiotic potential. For instance, the production of health-promoting conjugated linoleic acids (CLA) has been reported for *Bifidobacterium* strains,[50] *L. plantarum* JCM 1551,[51] and specific *L. acidophilus* strains. Also, conversion of phytoestrogen precursors to bioactive metabolites by supplemented microorganisms is a potential pathway for future probiotic applications. For example, Decroos et al. previously isolated a microbial consortium that converts soy-derived daidzein into the bioactive equol,[52] while Possemiers et al. performed an *in vitro* investigation of the probiotic potential of *Eubacterium limosum* strains to convert hop isoxanthohumol into 8-prenylnaringenin.[53]

Physiological functionality

Probiotic microorganisms have been reported to enhance GI transit. Hamilton-Miller previously reviewed such functionality for the application of probiotic products in elderly persons.[54] Other potential physiological effects may include the reduction by probiotics of bloating or gas production, the enhancement of ion absorption by intestinal epithelial cells[55] and the decrease of bile salt toxicity or the decrease of serum cholesterol levels by bile salt hydrolase positive probiotics.[56,57]

Lowering health detrimental components in the gut

Probiotic microorganisms are also applied to reduce the health risks from hazardous components. For example, oral exposure to contaminants, either from a food matrix or from an environmental matrix (soil, dust, water) is the most dominant scenario by which the human body gets internally exposed to contaminants. These can be: i) mycotoxins, produced from fungi on a wide variety of crops, cereals in particular; ii) xenobiotics with toxic properties as unwanted residues from environmental contamination of the food chain; or iii) hazardous compounds from the food production process as such (e.g. PAH production during grilling of meat). The mode of action by which these probiotics lower the risk derived from ingested hazardous components often relates to the sorption of the compound to microbial biomass. This is the case for aflatoxin B1, for example, which has been shown *in vitro* to be bound by probiotic strains.[58] Another mode of action may be direct detoxification of the hazardous compound, such as the breakdown of fumonisin by *Pediococcus pentosaecus* L006 isolated from corn leaves. A final mode of action is more indirect and resembles the abovementioned probiotic modulation of gut microenvironment, where (food) pathogens produce toxins. For example, the production of organic acids by probiotic microorganisms was reported to negatively affect the production of Shiga-toxin 2 from enterohemorrhagic *E. coli* O157:H7.

Immunological functionality

The immunological benefits of probiotics can be due to activation of local macrophages and modulation of IgA production locally and systemically, to changes in pro/anti-inflammatory cytokine profiles, or to the modulation of response towards food antigens.[59,60]

Probiotic products in prevention and treatment

The following paragraphs does not aim to provide a complete overview of all indications in which probiotics have been studied as possible preventive and/or therapeutic intervention, since new manuscripts are published weekly. Rather, the authors focused on the most relevant indications for children.

Acute infectious diarrhea

Probiotics have been largely studied for the prevention of acute infectious diarrhea. Large, randomized controlled trials (RCT) provide evidence of a very modest effect (statistically significant, but of questionable clinical importance) of some probiotic strains (*L. rhamnosus* GG, and strains of *L. reuteri* and *Bifidobacterium (B.) animalis* subsp. *lactis*) on the prevention of community-acquired diarrhea.[61–69] Many randomized and placebo-controlled trials conducted in different parts of the world have assessed the use of probiotics for prevention of diarrhea acquired in day-care centers. The main probiotics tested were *L. rhamnosus* GG, *B. animalis* subsp. *lactis* alone or in combination with *S. thermophilus*, and *L. reuteri*, *L. rhamnosus* (not *GG*), and *L. acidophilus* either alone or in a comparative study. The evidence of their efficacy in these settings is only modest for the prevention of diarrhea and sometimes also for preventing upper respiratory infections.[66] However, the protective effect on diarrhea prevention becomes far less significant if the incidence of diarrhea (episodes per patient-month) rather than the percentage of patients with diarrhea is taken into account.[68] In hospitalized children, the administration of *L. reuteri* DSM 17938 compared with placebo had no effect on the overall incidence of nosocomial diarrhea, including rotavirus infection.[70] Although the same strain prevented diarrhea in preschool children,[71] the clinical impact of such findings can be questioned.[72]

The use of the following probiotics (in alphabetical order) may be considered in the management of children with acute gastroenteritis in addition to rehydration therapy: *L. rhamnosus GG* (low quality of evidence; strong recommendation) and *S. boulardii* (low quality of evidence; strong recommendation). Less compelling evidence is available for

L. reuteri DSM 17938 (very low quality of evidence; weak recommendation) and heat-inactivated *L. acidophilus* LB (very low quality of evidence; weak recommendation).[73] The latter, although traditionally discussed with other probiotics, does not fit in the definition of probiotics. A number of RCTs have evaluated the effect of *Enterococcus faecium* SF6873.[73] A sub-group analysis performed within a Cochrane review (search date: July of 2010) found that *E. faecium* SF68 reduced the risk of diarrhea lasting ≥ four days (four RCTs, n = 333; RR: 0.21; 95% CI: 0.08 to 0.52).[73] However, *in vitro* studies have documented that the *E. faecium* SF68 strain is a possible recipient of vancomycin resistance genes.[74] Considering that the risk for *in vivo* conjugation cannot be discarded, probiotics with safety issues should not be used.[73] Recent publications have strengthened the evidence for *L. reuteri* in the treatment of diarrhea in hospitalized children.[75,76] Other strains or combinations of strains have been tested, but evidence of their efficacy is weak or preliminary. Mixtures of different strains do not necessarily score better.[77] Since in tropical countries with zinc-deficient regions, zinc is now added to oral rehydration salts (ORS), the impact of probiotics above its efficacy should be studied.[77] Considering that an acute gastroenteritis will heal spontaneously in almost every child, the cost/benefit impact will in great part determine whether probiotics should be used.[78,79]

Antibiotic-associated diarrhea (AAD)

The pooled relative risk in a meta-analysis of 63 RCTs, which included 11,811 participants, indicated a statistically significant association of probiotic administration with reduction in AAD (relative risk, 0.58; 95% CI, 0.50 to 0.68; p < 0.001; I(2), 54%; [risk difference, -0.07; 95% CI, -0.10 to -0.05], [NNT 13; 95% CI, 10.3 to 19.1])[80] Another meta-analysis concluded the NNT was 8.[81] According to a recent meta-analysis, probiotics significantly reduce the risk of AAD in children.[82] Preplanned subgroup analysis showed that reduction of the risk of AAD was associated with the use of *L. rhamnosus* GG (95% CI: 0.15 to 0.6), *S. boulardii* (95% CI: 0.07-0.6), or *B. lactis* and *S. thermophilus* (95% CI: 0.3 to 0.95).[82] For every seven patients that would develop diarrhea while being treated with antibiotics, one fewer will develop AAD if also receiving probiotics[82] Only *S. boulardii* was reported to be effective in *C. difficile* disease.[83–85] Recently, a large single-center study showed in elderly that *S. boulardii* was not effective in preventing the development of AAD or in prevention of *C. difficile* infection.[86] In many studies, there is no evidence to support the use of any (other) probiotic to prevent the recurrence of *C. difficile* infection or to treat existing *C. difficile* diarrhea.[64] A new meta-analysis concluded that probiotics significantly reduce the incidence of pediatric AAD (22 trials; RR = 0.42; 95% CI: 0.33-0.53) and the incidence of pediatric *C. difficile* infection (five trials; RR = 0.35; 95% CI: 0.13-0.92).[85] *S. boulardii* (RR = 0.43; 95% CI: 0.32-0.60) and *L. rhamnosus* GG (RR = 0.36; 95% CI: 0.19-0.69) are the two best studied strains.[87] In most studies, the probiotic is started together with antibiotic treatment.[88]

Traveler's diarrhea

Traveler's diarrhea is a frequent condition of great socioeconomic impact. In this topic, there are more reviews than original research studies published. Different RCTs have been performed evaluating the efficacy of probiotics in the prevention of traveler's diarrhea. One trial with *L. acidophilus* and two with *L. rhamnosus* GG observed negative results.[89–91] One trial with *S. boulardii* reported a small but significant preventive effect in a subgroup, suggesting geographical differences in efficacy.[92] In a review, McFarland concluded that there is comparable evidence for efficacy for *L. rhamnosus* GG, *L. casei* DN-114001, and *S. boulardii*, and no efficacy for *L. acidophilus*.[93] Since the number of studies in traveler's diarrhea is very limited, a recent meta-analysis concluded that probiotics are not efficient for traveler's diarrhea.[94] There are no data on prebiotics and prevention or treatment of traveler's diarrhea. Overall, the number of studies is too small to allow for recommendations.[95]

Irritable Bowel Syndrome (IBS)

There is substantial literature on the effect of probiotics on IBS in adults, but data in children are limited. A Cochrane review from 2009 failed to demonstrate an effect of fiber supplements and recorded a limited effect of lactobacilli on symptoms compared to placebo (OR: 1.17; 95% CI: 0.62, 2.21).[96]

A RCT of six weeks with *L. rhamnosus* GG *versus* placebo showed overall negative results in 50 children and young adults, although there was a lower incidence of perceived abdominal distension in the *L. rhamnosus* GG group.[97] *L. rhamnosus* GG, but not placebo, caused a significant reduction of both frequency and severity of abdominal pain compared to baseline, and influenced intestinal permeability testing.[98] A meta-analysis demonstrated that, compared with placebo, *L. rhamnosus* GG supplementation was associated with a significantly higher rate of treatment responders in the overall population with abdominal pain-related functional GI disorders and in the IBS subgroup.[99] However, no difference was observed in children with functional abdominal pain or functional dyspepsia who received placebo or *L. rhamnosus* GG. A randomized cross-over trial with VSL#3 and placebo, comprising 59 patients for six weeks, with a two-week washout period in-between, showed a superior effect of VSL#3 compared to placebo in symptom relief, as well as in abdominal pain/discomfort, abdominal bloating/gassiness, and family assessment of life disruption.[99] No significant difference was found in the stool pattern.[100]

There are no data on prevention or treatment of IBS with prebiotics. Data from one trial suggest that, in infants, a prebiotic-containing whey-based formula provides superior GI comfort than a control formula.[101] A peptide-based formula containing fiber was as well-tolerated as a fiber-free formula in a small population of children with GI impairments.[102] Extremes of stool consistency were normalized with the fiber formula. No significant differences were observed in vomiting, abdominal pain, feeding intakes, or weight gain between the two formulas.[103] Synbiotics should be further investigated in this indication.[103] Probiotics are

more effective than placebo in the treatment of patients with abdominal pain-related functional gastro-intestinal disorders, especially with respect to patients with IBS.[104]

Helicobacter pylori

The use of probiotics in *H. pylori*-colonized subjects with gastric inflammation is supported by many observations. Specific strains of *Lactobacillus* and *Bifidobacerium* exert *in vitro* bactericidal effects against *H. pylori* through the release of bacteriocins or production of organic acids, and/or inhibit its adhesion to epithelial cells. Such protective effects have been confirmed in animal models. Clinical trials are very important, since *in vitro* results cannot always be reproduced in patients. Probiotics decrease the bacterial load and improve the immune response.[105] Results of clinical trials indicate that probiotics generally do not eradicate *H. pylori*, but decrease the density of colonization, thereby maintaining lower levels of this pathogen in the stomach; in association with antibiotic treatments, some probiotics increased eradication rates and/or decreased adverse effects due to the antibiotics. Many studies show a moderate higher eradication rate (~10%) of *H. pylori* when probiotics are added to the antibiotics and proton pump inhibitor.[106] Although *L. rhamnosus* GG appears not to improve eradication,[107] most probiotic bacteria and yeasts reduce the adverse effects of standard *H. pylori* eradication regimens.[108,109] Probiotics supplementation in triple therapy for *H. pylori* infection may have beneficial effects on eradication and therapy-related side effects, particularly diarrhea, in children.[110]

Constipation

Constipation is a frequent problem in childhood in which pre- and probiotics could have a positive influence on the intestinal microbiota with an effect on stool consistency and frequency. Unfortunately, study results are contradictory. In an open trial, *B. breve* was effective in increasing stool frequency in children with functional constipation.[111] Furthermore, it had a positive effect regarding improving stool consistency, decreasing the number of fecal incontinence episodes, and reducing abdominal pain.[111] In another open trial, a probiotic mixture (Ecologic Relief®, Winclove Pro Biotics, Netherlands) containing *B. bifidum*, *B. infantis*, *B. longum*, *L. casei*, *L. plantarum*, and *L. rhamnosus* showed positive effects on constipation symptoms.[112] *L. rhamnosus* Lcr35 was effective in treating children with chronic constipation.[113] *B. lactis* was reported to be non effective for constipation.[94,114] *L. reuteri* DSM 17938 had a positive effect in infants with chronic constipation on bowel frequency, even when there was no improvement in stool consistency and episodes of inconsolable crying episodes.[115] A Brazilian study showed a positive influence of yoghurt on stool frequency with an additional effect of yoghurt supplemented with *B. longum*.[116] In constipated children, a fermented dairy product containing *B. animalis* subsp. *lactis* DN-173 010 increase stool frequency, but this increase was comparable with that observed in the control group.[117] There is currently not sufficient evidence to recommend fermented dairy products containing strain DN-173 010 in this category of patients.[117] No evidence for any effect was found for fluid supplements, prebiotics, probiotics, or behavioral intervention.[118] Probiotics have not proven effective for children with functional constipation.[104] Until more data are available, the use of probiotics for the treatment of constipation condition should be considered investigational.[119]

Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a severe condition occurring especially in preterm infants. Abnormal GI microbiota development has been hypothesized as one of the possible etiologic factors. The first publication reporting that *L. acidophilus* and *B. infantis* reduced NEC dates back from 1999.[120] This was followed by a negative study showing that seven days of *L. rhamnosus* GG supplementation starting with the first feed was not effective in reducing the incidence of urinary tract infection, **NEC** and sepsis in preterm infants.[121] Then, several randomized trials with different lactobacilli and bifidobacteria showed a significant reduction in development of NEC.[122,123] Although *S. boulardii* was shown to ameliorate hypoxia/reoxygenation-induced NECs in young mice,[124] it did not protect for NEC in infants.[125] A Cochrane review concluded in 2008 that enteral probiotic supplementation reduced the incidence of NEC stage II or more and mortality.[126] No systemic infections or serious adverse events were directly attributed to the administered probiotic microorganism.[126] According to the published trials, the NNT to prevent one case of NEC is 21 and 27.[126] However, the centers in which these trails have been performed have a much higher incidence of NEC than most European or North American centers. The recommendation may be different in centers with a high incidence of NEC, in which other measurements to decrease NEC are difficult to apply. The updated Cochrane review from 2011 comes to different conclusions: enteral supplementation of **probiotics** prevents severe **NEC** and all cause mortality in preterm infants.[127] The updated review of available evidence supports a change in practice. More studies are needed to assess efficacy in extremely low birth weight infants and to assess the most effective formulation and dose to be used.[127] The debate regarding whether probiotics should be systematically given to preterms is still ongoing. The 2012 systematic review of the American Pediatric Surgical Association Outcomes and Clinical Trials Committee acknowledges that recent **Cochrane** reviews support the use of prophylactic **probiotics** in preterm infants weighing less than 2,500 g to reduce the incidence of NEC, as well as the use of human breast milk rather than formula when possible. There is no clear evidence to support delayed initiation or slow advancement of feeds.[128] However, an expert group of nutritionists and neonatologists concluded that there is insufficient evidence to recommend the routine use of probiotics to decrease NEC.[129] According to this group, there is encouraging data to justify further investigation regarding the efficacy and safety of specific probiotics in circumstances of high local incidence of severe **NEC**.[129] According to others, available evidence is still too limited in order to

recommend probiotics to reduce NEC.[130] A third group suggests that it may become unethical not to give probiotics to preterm babies to decrease NEC.[131] Enteral supplementation of **probiotics** prevents severe NEC and all cause mortality in preterm infants.[132]

Colic

Colic is a frequent problem in infants and often parents are desperate for a solution. In this indication, the effect of *L. reuteri* has been exhaustively studied in breastfed infants.[133–135] However, recent data suggested that infants given the same probiotic cry for 50 minutes more than those given placebo.[136] Dupont et al. reported efficacy of another probiotic strain in formula fed infants.[137] A synbiotic sachet containing 1 billion CFU of *L. casei*, *L. rhamnosus*, *S. thermophilus*, *B. breve*, *L. acidophilus*, *B.infantis*, *L. delbrueckii* subsp. *bulgaricus*, and fructooligosacharide was shown to be effective in reducing colic in breastfed infants when compared to placebo.[138]

Allergy and atopic dermatitis

Simultaneous pro- and prebiotic treatment (a mixture of four strains and GOS) given to pregnant women for two to four weeks before delivery and to the infants for six months showed no effect on the cumulative incidence of allergic diseases at the age of 2 years when compared with placebo, but tended to reduce IgE-associated (atopic) diseases since a significant reduction of (atopic) eczema was noticed.[139] However, Taylor et al challenged the role of probiotics in allergy prevention, observing that early probiotic supplementation with *L. acidophilus* did not reduce the risk of atopic dermatitis (AD) in high-risk infants, and was even associated with increased allergen sensitization in infants receiving supplements.[140] A Cochrane review from 2007 concluded that there was insufficient evidence to recommend the addition of probiotics to infant feeds for prevention of allergic disease or food hypersensitivity.[141] Although there was a reduction in clinical eczema in infants, this effect was not consistent between studies, and caution was advised in view of methodological concerns regarding the included studies.[142] However, the efficacy of probiotic intervention to reduce atopic dermatitis and/or allergic disease may depend on the moment of intervention. Preventive administration of probiotics may be only effective if given during pregnancy. Probiotics given to unselected mothers reduced the cumulative incidence of AD, but had no effect on atopic sensitization.[142] A recent meta-analysis showed that the administration of lactobacilli during pregnancy prevented atopic eczema in children aged 2 to 7 years.[143] However, a mixture of various bacterial strains does not affect the development of atopic eczema, independent of whether they contain lactobacilli or not.[143] *L. rhamnosus* HN001 was reported effective against eczema in the first two years of life, persisting to age 4 years, while *B. animalis* subsp. *lactis HN019* had no effect.[144] Therefore, not only timing of administration appears to important, but also strain specificity. However, timing of administration and strain specificity were then again contradicted in the meta-analysis by Pelucchi et al., supporting a moderate role of probiotics in the prevention of atopic dermatitis and IgE-associated atopic dermatitis in infants, regardless of the time of probiotic use (pregnancy or early life) or the subject(s) receiving probiotics (mother, child, or both).[145] The data on probiotics and allergy need further clarification, because data are somehow contradictory. Geographical or genetic differences may have a detrimental role, especially for atopic dermatitis.

Ninety infants with atopic dermatitis, age < 7 months, were randomized to receive an infant formula with *B. breve* M-16 V and a mixture of short chain GOS and long chain FOS, or the same formula without synbiotics during 12 weeks.[146] There were no significant differences between the synbiotic and the placebo group.[146] The same group showed that synbiotics prevent asthma-like symptoms in infants with AD.[147] At the same time, another group reported that a synbiotic combination of *L. salivarius* plus FOS is superior to the prebiotic alone for treating moderate to severe childhood AD.[148] While some studies with probiotics as a treatment for AD show a benefit,[149] most studies are negative. No benefit was reported from supplementation with *B. animalis* subsp. *lactis* or *L. paracasei* in the treatment of eczema, when given as an adjunct to basic topical treatment, and no effect on the progression of allergic disease from age 1 to 3 years was observed.[150] Most reviews conclude that probiotics are not effective in reducing atopic dermatitis. These contradictory results suggest strain specificity or a genetic influence on the efficacy of probiotics in children with atopic dermatitis. A review of 13 studies of probiotics for treating established eczema did not show convincing evidence of a clinically worthwhile benefit.[151] However, according to a recent meta-analysis, the overall result suggests that probiotics could be an option for the treatment of atopic dermatitis, especially for moderate to severe cases, and no evidence was found supporting the beneficial role of probiotics in infants.[152]

Extra-intestinal infections and other effects

No pediatric studies have demonstrated definite beneficial effects of administering probiotics to treat extra-intestinal infections such as respiratory tract infections or otitis media.[88,153] There is no evidence that probiotics decrease extra-intestinal infections. There is some evidence that some lactobacilli might prevent recurrent urinary tract infection in women. However, data in children are lacking. The same is true for recurrent vulvovaginitis. Sazawal et al. showed that prebiotic and probiotic fortified milk prevented morbidities among children in a community-based RCT.[154]

Candidiasis accounts for 10% to 20% of bloodstream infections in pediatric intensive care units (PICUs) and a significant increase in morbidity, mortality, and length of hospital stay.[155] A few studies have demonstrated that probiotics are able to prevent *Candida* outgrowth and colonization in neonates, whereas their role in preventing invasive candidiasis in such patients is still unclear.[155]

Purified phytases from *B. longum* subsp. *infantis* and *B. pseudocatenulatum* reduced the contents of phytate when compared to control samples (untreated or treated with fungal phytase), and led to increased levels of myo-inositol

triphosphate.[156] This is the first example of the application of purified bifidobacterial phytases in food processing, demonstrating the potential of these enzymes to be used in products for human consumption.[156] Lactic acid bacteria improve the synthesis of vitamins B2, B11 and B12 and have the potential strategies to increase B-group vitamin content in cereals-based products.[157] Vitamin-producing *L.* has been leading to the elaboration of novel fermented functional foods.[157]

Pandemic obesity is now a matter of interest in all developed and developing countries. Treatment with probiotics selectively changes the composition of the gut microbiota in favor of specific genus and even strains. Few intervention studies with probiotics in overweight or obese individuals have been published until now, and mostly focus on *L.* or *B.* The administration of a strain of *L. gasseri* in obese and type 2 diabetic patients has been shown to decrease fat mass (visceral and subcutaneous) and body mass index.[158] In addition, Andreasen et al. have demonstrated that the administration of *Lactobacillus spp.* positively impact on insulin sensitivity.[159] Compelling evidence suggests that early gut microbiota modulation with probiotics reduces the body mass index in young children by restraining excessive weight gain during the first years of life (from 0 to 10 years of follow-up).[160] So far, only few data are available on the possible application of lactobacilli or bifidobacteria to counteract adiposity.

Fecal microbiota transplantation

A new approach in microbial therapeutic applications is transplantation of intestinal microbiota, especially in difficult to treat conditions in which it is known that the fecal microbiota is abnormal.[161,162] Observed side effects warrant caution in the ongoing pursuit of this treatment option.[162] There is evidence that many diseases are related to intestinal dysbiosis. As a consequence, manipulation of the intestinal microbiota is a very attractive therapeutic approach. However, results are often negative,[162] although positive results have been reported.[163] The transplanted microbiota should be carefully screened for pathogens.[164] Bacteremia has been reported as an adverse event.[165] However, the first cure of early onset colitis after fecal microbiota transplantation has been reported.[166] Further studies should now focus on the reasons for success and failure.

Safety and side effects

Probiotics have a long record of safety, which relates primarily to the use of lactobacilli and bifidobacteria.[167] Experience with other microorganisms used as probiotic is more limited. There is no such thing as zero risk, particularly in the context of certain forms of host susceptibility.[167] Probiotics are generally regarded as safe, and side effects in ambulatory care have almost not been reported. Large scale epidemiological studies in countries where probiotic use is endemic demonstrate (in adults) low rates of systemic infection, between 0.05 and 0.40%.[168] Administration during pregnancy and early infancy is considered safe.[169] Probiotic compounds may contain hidden allergens of food and may not be safe for subjects with allergy to cow's milk or hen's eggs.[170] Documented invasive infections have been primarily noted to occur in immunocompromised adults, but invasive infections in infants and children are extremely rare.[171–173] Two cases of bacteremia attributable to *Lactobacillus* supplementation with genotypically identical clinical and supplement isolates were recently reported in an infant and a child without underlying GI disease or immunocompromised status.[174] Sepsis with probiotic lactobacilli has been reported in children with short gut. Recently, plasmid transfer of antibiotic resistance has been shown to be clinically possible. Long-term use of probiotics under antibiotic selection pressure could cause antibiotic resistance, and the resistance gene could be transferred to other bacteria.[175] Translocation from the gastro-intestinal tract into the systemic circulation has not been reported. There is poor public understanding of the concept of risk, in general, and risk/benefit analysis, in particular.[168] Uncertainty regarding the potential for transfer of antibiotic resistance with probiotics persists, but the risk appears to be low with currently available probiotic products.[168] As with other forms of therapeutics, the safety of probiotics should be considered on a strain-by-strain basis.[168] The potential benefits of supplementation should be weighed against the risk of development of an invasive infection resulting from probiotic therapy.

Conclusion

Probiotics have entered the mainstream of healthcare. The gastro-intestinal microbiota is fundamental for the development of the immune system. Although the main indications of the medical use of probiotics is still in the area of the prevention and treatment of gastro-intestinal related disorders, gradually more evidence is collected on extra-intestinal indications, such as vaginitis, atopic dermatitis, and respiratory tract infections. Randomized controlled trials with commercially available products in the claimed indications are mandatory before their use can be recommended. Currently, *L. rhamnosus* GG and *S. boulardii* are the best-studied strains, while recent literature provides positive data on *L. reuteri*. Although adverse effects have been sporadically reported, probiotics can be considered safe. Misuse and use of products that have not been validated may constitute potential drawbacks.

Conflicts of interest

Yvan Vandenplas is consultant for Biocodex and United Pharmaceuticals. The co-authors did not report any potential conflicts of interest.

References

1. Food and Agriculture Organization (FAO). World Health Organization (WHO). Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties

of probiotics in food including powder milk with live lactic acid bacteria. 1-4 October 2001, Córdoba, Argentina. [cited 18 Aug 2014]. Available from: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf
2. Food and Agriculture Organization (FAO). World Health Organization (WHO). Report of a Joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. April 30, May 1; London Ontario, Canada; 2002. [cited 18 Aug 2014]. Available from: ftp://ftp.fao.org/es/esn/food/wgreport2.pdf.
3. Ashwell M. Concepts of functional foods. Brussels. International Life Sciences Institute (ILSI) Europe. 2002.
4. European Food and Feed Cultures Association (EFFCA). Definition of microbial food culture (MFC). Brussels: EFFCA; 2003.
5. Vandenplas Y. Author's reply: Identification of probiotics by specific strain name. Aliment Pharmacol Ther. 2012;35:860.
6. European Parliament and Council. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union. OJ L 404, 30.12. 2006. Corrigendum OJ L 12, 18.1.2007, p. 3-18. [cited 18 Aug 2014]. Available from: ttps://www.fsai.ie/uploadedFiles/Cor_Reg1924_2006.pdf.
7. Huys G, Vancanneyt M, D'Haene K, Vankerckhoven V, Goossens H, Swings J. Accuracy of species identity of commercial bacterial cultures intended for probiotic or nutritional use. Res Microbiol. 2006;157:803–10.
8. Sanders ME. Probiotics: considerations for human health. Nutr Rev. 2003;61:91–9.
9. Stamatova I, Meurman JH. Probiotics: health benefits in the mouth. Am J Dent. 2009;22:329–38.
10. Meurman JH. Probiotics: do they have a role in oral medicine and dentistry? Eur J Oral Sci. 2005;113:188–96.
11. Hoesl CE, Altwein JE. The probiotic approach: an alternative treatment option in urology. Eur Urol. 2005;47:288–96.
12. Barrons R, Tassone D. Use of *Lactobacillus* probiotics for bacterial genitourinary infections in women: a review. Clin Ther. 2008;30:453–68.
13. Reid G, Bocking A. The potential for probiotics to prevent bacterial vaginosis and preterm labor. Am J Obstet Gynecol. 2003;189:1202–8.
14. Falagas ME, Betsi GI, Tokas T, Athanasiou S. Probiotics for prevention of recurrent urinary tract infections in women: a review of the evidence from microbiological and clinical studies. Drugs. 2006;66:1253–61.
15. Hacini-Rachinel F1, Gheit H, Le Luduec JB, Dif F, Nancey S, Kaiserlian D. Oral probiotic control skin inflammation by acting on both effector and regulatory T cells. PLoS One. 2009;4:e4903.
16. Caramia G1, Atzei A, Fanos V. Probiotics and the skin. Clin Dermatol. 2008;26:4–11.
17. Hojsak I, Abdović S, Szajewska H, Milosević M, Krznarić Z, Kolacek S. *Lactobacillus* G.G. in the prevention of nosocomial gastrointestinal and respiratory tract infections. Pediatrics. 2010;125:e1171–7.
18. Possemiers S, Marzorati M, Verstraete W, Van de Wiele T. Bacteria and chocolate: a successful combination for probiotic delivery. Int J Food Microbiol. 2010;141:97–103.
19. Pereg D, Kimhi O, Tirosh A, Orr N, Kayouf R, Lishner M. The effect of fermented yogurt on the prevention of diarrhea in a healthy adult population. Am J Infect Control. 2005;33:122–5.
20. Magalhães KT, Pereira MA, Nicolau A, Dragone G, Domingues L, Teixeira JA, et al. Production of fermented cheese whey-based beverage using kefir grains as starter culture: evaluation of morphological and microbial variations. Bioresour Technol. 2010;101:8843–50.
21. Steidler L, Hans W, Schotte L, Neirynck S, Obermeier F, Falk W, et al. Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. Science. 2000;289:1352–5.
22. Foligne B, Dessein R, Marceau M, Poiret S, Chamaillard M, Pot B, et al. Prevention and treatment of colitis with *Lactococcus lactis* secreting the immunomodulatory *Yersinia* LcrV protein. Gastroenterology. 2007;133:862–74.
23. Kumar M, Nagpal R, Verma V, Kumar A, Kaur N, Hemalatha R, et al. Probiotic metabolites as epigenetic targets in the prevention of colon cancer. Nutr Rev. 2013;71:23–34.
24. Huseini HF, Rahimzadeh G, Fazeli MR, Mehrazma M, Salehi M. Evaluation of wound healing activities of kefir products. Burns. 2012;38:719–23.
25. Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV. Microencapsulation of probiotics for gastrointestinal delivery. J Control Release. 2012;162:56–67.
26. Kumar M, Nagpal R, Kumar R, Hemalatha R, Verma V, Kumar A, et al. Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Exp Diabetes Res. 2012;2012:902917.
27. Van den Abbeele P, Grootaert C, Possemiers S, Verstraete W, Verbeken K, et al. *In vitro* model to study the modulation of the mucin-adhered bacterial community. Appl Microbiol Biotechnol. 2009;83:349–59.
28. Macfarlane S, Furrie E, Kennedy A, Cummings JH, Macfarlane GT. Mucosal bacteria in ulcerative colitis. Br J Nutr. 2005;93:S67–72.
29. Chassaing B, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. Gastroenterology. 2011;140:1720–8.
30. Lebeer S, Claes I, Tytgat HL, Verhoeven TL, Marien E, von Ossowski I, et al. Functional analysis of *Lactobacillus rhamnosus* GG pili in relation to adhesion and immunomodulatory interactions with intestinal epithelial cells. Appl Environ Microbiol. 2012;78:185–93.
31. Nagao F, Nakayama M, Muto T, Okumura K. Effects of a fermented milk drink containing *Lactobacillus casei* strain Shirota on the immune system in healthy human subjects. Biosci Biotechnol Biochem. 2000;64:2706–8.
32. Hatakka K, Savilahti E, Pönkä A, Meurman JH, Poussa T, Näse L, et al. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. BMJ. 2001;322:1327.
33. Makino S, Ikegami S, Kume A, Horiuchi H, Sasaki H, Orii N. Reducing the risk of infection in the elderly by dietary intake of yoghurt fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1. Br J Nutr. 2010;104:998–1006.
34. Twetman S, Stecksén-Blicks C. Probiotics and oral health effects in children. Int J Paediatr Dent. 2008;18:3–10.
35. Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä A, Poussa T, et al. Effect of long-term consumption of a probiotic bacterium *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. Caries Res. 2001;35:412–20.
36. Sarantos SR. The importance of probiotic supplementation in conjunction with orthodontic therapy. J N J Dent Assoc. 2006;77:10–3.
37. Reid G, Beuerman D, Heinemann C, Bruce AW. Probiotic *Lactobacillus* dose required to restore and maintain a normal vaginal flora. FEMS Immunol Med Microbiol. 2001;32:37–41.
38. Rossi A, Rossi T, Bertini M, Caccia G. The use of *Lactobacillus rhamnosus* in the therapy of bacterial vaginosis Evaluation

of clinical efficacy in a population of 40 women treated for 24 months. Arch Gynecol Obstet. 2010;281:1065–9.
39. Andreeva P, Dimitrov A. The probiotic *Lactobacillus acidophilus* – an alternative treatment of bacterial vaginosis. Akush Ginekol (Sofiia). 2002;41:29–31.
40. Cats A, Kuipers EJ, Bosschaert MA, Pot RG, Vandenbroucke-Grauls CM, Kusters JG. Effect of frequent consumption of a *Lactobacillus casei*-containing milk drink in *Helicobacter pylori*-colonized subjects. Aliment Pharmacol Ther. 2003;17:429–35.
41. Ushiyama A, Tanaka K, Aiba Y, Shiba T, Takagi A, Mine T, et al. *Lactobacillus gasseri* OLL2716 as a probiotic in clarithromycin-resistant *Helicobacter pylori* infection. J Gastroenterol Hepatol. 2003;18:986–91.
42. Collado MC, González A, González R, Hernández M, Ferrús MA, Sanz Y. Antimicrobial peptides are among the antagonistic metabolites produced by *Bifidobacterium* against *Helicobacter pylori*. Int J Antimicrob Agents. 2005;25:385–91.
43. Alfaleh K, Anabrees J, Bassler D. Probiotics reduce the risk of necrotizing enterocolitis in preterm infants: a meta-analysis. Neonatology. 2010;97:93–9.
44. Weber TK, Polanco I. Gastrointestinal microbiota and some children diseases: a review. Gastroenterol Res Pract. 2012;2012:676585.
45. Santos F, Vera JL, van der Heijden R, Valdez G, de Vos WM, Sesma F, et al. The complete coenzyme B12 biosynthesis gene cluster of *Lactobacillus reuteri* CRL1098. Microbiology. 2008;154:81–93.
46. Fabian E, Majchrzak D, Dieminger B, Meyer E, Elmadfa I. Influence of probiotic and conventional yoghurt on the status of vitamins B1 B2 and B6 in young healthy women. Ann Nutr Metab. 2008;52:29–36.
47. de Vrese M, Stegelmann A, Richter B, Fenselau S, Laue C, Schrezenmeir J. Probiotics –compensation for lactase insufficiency. Am J Clin Nutr. 2001;73:421S–9S.
48. Sousa T, Paterson R, Moore V, Carlsson A, Abrahamsson B, Basit AW. The gastrointestinal microbiota as a site for the biotransformation of drugs. Int J Pharm. 2008;363:1–25.
49. Fischbach MA. Antibiotics from microbes: converging to kill. Curr Opin Microbiol. 2009;12:520–7.
50. Gorissen L, Raes K, Weckx S, Dannenberger D, Leroy F, De Vuyst L, et al. Production of conjugated linoleic acid and conjugated linolenic acid isomers by *Bifidobacterium* species. Appl Microbiol Biotechnol. 2010;87:2257–66.
51. Ando A, Ogawa J, Kishino S, Shimizu S. CLA production from ricinoleic acid by lactic acid bacteria. J Am Oil Chem Soc. 2003;80:889–94.
52. Decroos K, Vanhemmens S, Cattoir S, Boon N, Verstraete W. Isolation and characterisation of an equol-producing mixed microbial culture from a human faecal sample and its activity under gastrointestinal conditions. Arch Microbiol. 2005;183:45–55.
53. Possemiers S, Rabot S, Espín JC, Bruneau A, Philippe C, González-Sarrías A, et al. *Eubacterium limosum* activates isoxanthohumol from hops (*Humuluslupulus* L.) into the potent phytoestrogen 8-prenylnaringenin *in vitro* and in rat intestine. J Nutr. 2008;138:1310–6.
54. Hamilton-Miller JM. Probiotics and prebiotics in the elderly. Postgrad Med J. 2004;80:447–51.
55. Borthakur A, Gill RK, Tyagi S, Koutsouris A, Alrefai WA, Hecht GA, et al. The probiotic *Lactobacillus acidophilus* stimulates chloride/hydroxyl exchange activity in human intestinal epithelial cells. J Nutr. 2008;138:1355–9.
56. De Boever P, Wouters R, Verschaeve L, Berckmans P, Schoeters G, Verstraete W. Protective effect of the bile salt hydrolase-active *Lactobacillus reuteri* against bile salt cytotoxicity. Appl Microbiol Biotechnol. 2000;53:709–14.
57. De Smet I, De Boever P, Verstraete W. Cholesterol lowering in pigs through enhanced bacterial bile salt hydrolase activity. Br J Nutr. 1998;79:185–94.
58. Gratz S, Mykkänen H, Ouwehand AC, Juvonen R, Salminen S, El-Nezami H. Intestinal mucus alters the ability of probiotic bacteria to bind aflatoxin B1 *in vitro*. Appl Environ Microbiol. 2004;70:6306–8.
59. Kabeerdoss J, Devi RS, Mary RR, Prabhavathi D, Vidya R, Mechenro J, et al. Effect of yoghurt containing *Bifidobacterium lactis* Bb12® on faecal excretion of secretory immunoglobulin A and human beta-defensin 2 in healthy adult volunteers. Nutr J. 2011;10:138.
60. Ghadimi D, Fölster-Holst R, de Vrese M, Winkler P, Heller KJ, Schrezenmeir J. Effects of probiotic bacteria and their genomic DNA on TH1/TH2-cytokineproduction by peripheral blood mononuclear cells (PBMCs) of healthy and allergic subjects. Immunobiology. 2008;213:677–92.
61. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding *of Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet. 1994;344:1046–9.
62. Szajewska H, Kotowska M, Mrukowicz JZ, Armańska M, Mikołajczyk W. Efficacy of *Lactobacillus* GG in prevention of nosocomial diarrhea in infants. J Pediatr. 2001;138:361–5.
63. Mastretta E, Longo P, Laccisaglia A, Balbo L, Russo R, Mazzaccara A, et al. Effect of *Lactobacillus* GG and breast-feeding in the prevention of rotavirus nosocomial infection. J Pediatr Gastroenterol Nutr. 2002;35:527–31.
64. Szajewska H, Setty M, Mrukowicz J, Guandalini S. Probiotics in gastrointestinal diseases in children: hard and not-so-hard evidence of efficacy. J Pediatr Gastroenterol Nutr. 2006;42:454–75.
65. Agustina R, Kok FJ, van de Rest O, Fahmida U, Firmansyah A, Lukito W, et al. Randomized trial of probiotics and calcium on diarrhea and respiratory tract infections in Indonesian children. Pediatrics. 2012;129:e1155–64.
66. Guandalini S. Probiotics for prevention and treatment of diarrhea. J Clin Gastroenterol. 2011;45:S149–53.
67. Szajewska H, Mrukowicz JZ. Use of probiotics in children with acute diarrhea. Paediatr Drugs. 2005;7:111–22.
68. Chouraqui JP, Van Egroo LD, Fichot MC. Acidified milk formula supplemented with *Bifidobacterium lactis*: impact on infant diarrhea in residential care settings. J Pediatr Gastroenterol Nutr. 2004;38:288–92.
69. Thibault H, Aubert-Jacquin C, Goulet O. Effects of long-term consumption of a fermented infant formula (with *Bifidobacterium breve* c50 and *Streptococcus thermophilus* 065) on acute diarrhea in healthy infants. J Pediatr Gastroenterol Nutr. 2004;39:147–52.
70. Wanke M, Szajewska H. Lack of an effect of *Lactobacillus reuteri* DSM 17938 in preventing nosocomial diarrhea in children: a randomized, double-blind, placebo-controlled trial. J Pediatr. 2012;161:40–3.
71. Gutierrez-Castrellon P, Lopez-Velazquez G, Diaz-Garcia L, Jimenez-Gutierrez C, Mancilla-Ramirez J, Estevez-Jimenez J, et al. Diarrhea in preschool children and *Lactobacillus reuteri*: a randomized controlled trial. Pediatrics. 2014;133:e904–9.
72. Vandenplas Y. *Lactobacillus reuteri* is an effective option for the prevention of diarrhoea in preschool children but

may not be cost-effective in all settings. Evid Based Med. 2014.
73. Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Shamir R, et al. Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr. 2014;58:531–9.
74. Lund B, Edlund C. Probiotic *Enterococcus faecium* strain is a possible recipient of the vanA gene cluster. Clin Infect Dis. 2001;32:1384–5.
75. Dinleyici EC, PROBAGE Study Group. Vandenplas Y. Lactobacillus reuteri DSM 17938 effectively reduces the duration of acute diarrhoea in hospitalised children. Acta Paediatr. 2014;103:e300–5.
76. Szajewska H, Urbańska M, Chmielewska A, Weizman Z, Shamir R. Meta-analysis: *Lactobacillus reuteri* strain DSM 17938 (and the original strain ATCC 55730) for treating acute gastroenteritis in children. Benef Microbes. 2014;5:285–93.
77. Hegar B, Waspada IM, Gunardi H, Vandenplas Y. A double blind randomized trial showing probiotics to be ineffective in acute diarrhea in Indonesian children. Indian J Pediatr. 2014 [Epub ahead of print].
78. Vandenplas Y, De Hert S. Probiotical study group Cost/benefit of synbiotics in acute infectious gastroenteritis: spend to save. Benef Microbes. 2012;3:189–94.
79. Phavichitr N, Puwdee P, Tantibhaedhyangkul R. Cost-benefit analysis of the probiotic treatment of children hospitalized for acute diarrhea in Bangkok. Thailand Southeast Asian J Trop Med Public Health. 2013;44:1065–71.
80. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JN, Shanman R, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA. 2012;307:1959–69.
81. Videlock EJ, Cremonini F, Meta-analysis:. probiotics in antibiotic-associated diarrhoea. Aliment Pharmacol Ther. 2012;35:1355–69.
82. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. Lancet Infect Dis. 2006;6:374–82.
83. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. Am J Gastroenterol. 2006;101:812–22.
84. Johnston BC, Goldenberg JZ, Vandvik PO, Sun X, Guyatt GH. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database Syst Rev. 2011;(11):CD004827.
85. Tung JM, Dolovich LR, Lee CH. Prevention of *Clostridium difficile* infection with *Saccharomyces boulardii*: a systematic review. Can J Gastroenterol. 2009;23:817–21.
86. Pozzoni P, Riva A, Bellatorre AG, Amigoni M, Redaelli E, Ronchetti A, et al. *Saccharomyces boulardii* for the prevention of antibiotic-associated diarrhea in adult hospitalized patients: a single-center, randomized, double-blind, placebo-controlled trial. Am J Gastroenterol. 2012;107:922–31.
87. McFarland LV, Goh S. Preventing pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections with probiotics: a meta-analysis. World J Meta-Anal. 2013;1:102–20.
88. Braegger C, Chmielewska A, Decsi T, Kolacek S, Mihatsch W, Moreno L, et al. Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr. 2011;52:238–50.
89. Katelaris PH, Salam I, Farthing MJ. *Lactobacilli* to prevent traveler's diarrhea? N Engl J Med. 1995;333:1360–1.
90. Hilton E, Kolakowski P, Singer C, Smith M. Efficacy of *Lactobacillus* GG as a diarrheal preventive in travelers. J Travel Med. 1997;4:41–3.
91. Oksanen PJ, Salminen S, Saxelin M, Hämäläinen P, Ihantola-Vormisto A, Muurasniemi-Isoviita L, et al. Prevention of travellers' diarrhoea by *Lactobacillus* GG. Ann Med. 1990;22:53–6.
92. Kollaritsch H. Traveller's diarrhea among Austrian tourists to warm climate countries: II Clinical features. Eur J Epidemiol. 1989;5:355–62.
93. McFarland LV. Probiotics and diarrhea. Ann Nutr Metab. 2010;57:10–1.
94. Ibnou-Zekri N, Blum S, Schiffrin EJ, von der Weid T. Divergent patterns of colonization and immune response elicited from two intestinal *Lactobacillus strains* that display similar properties in vitro. Infect Immun. 2003;71:428–36.
95. DuPont HL, Ericsson CD, Farthing MJ, Gorbach S, Pickering LK, Rombo L, et al. Expert review of the evidence base for prevention of travelers' diarrhea. J Travel Med. 2009;16:149–60.
96. Huertas-Ceballos AA, Logan S, Bennett C, Macarthur C. Dietary interventions for recurrent abdominal pain (RAP) and irritable bowel syndrome (IBS) in childhood. Cochrane Database Syst Rev. 2009;(1):CD003019.
97. Bausserman M, Michail S. The use of *Lactobacillus* GG in irritable bowel syndrome in children: a double-blind randomized control trial. J Pediatr. 2005;147:197–201.
98. Francavilla R, Miniello V, Magistà AM, De Canio A, Bucci N, Gagliardi F, et al. A randomized controlled trial of *Lactobacillus* GG in children with functional abdominal pain. Pediatrics. 2010;126:e1445–52.
99. Horvath A, Dziechciarz P, Szajewska H. Meta-analysis: *Lactobacillus* rhamnosus GG for abdominal pain-related functional gastrointestinal disorders in childhood. Aliment Pharmacol Ther. 2011;33:1302–10.
100. Guandalini S, Magazzù G, Chiaro A, La Balestra V, Di Nardo G, Gopalan S, et al. VSL#3 improves symptoms in children with irritable bowel syndrome: a multicenter, randomized, placebo-controlled, double-blind, crossover study. J Pediatr Gastroenterol Nutr. 2010;51:24–30.
101. Vivatvakin B, Mahayosnond A, Theamboonlers A, Steenhout PG, Conus NJ. Effect of a whey-predominant starter formula containing LCPUFAs and oligosaccharides (FOS/GOS) on gastrointestinal comfort in infants. Asia Pac J Clin Nutr. 2010;19:473–80.
102. Khoshoo V, Sun SS, Storm H. Tolerance of an enteral formula with insoluble and prebiotic fiber in children with compromised gastrointestinal function. J Am Diet Assoc. 2010;110:1728–33.
103. Piirainen L, Kekkonen RA, Kajander K, Ahlroos T, Tynkkynen S, Nevala R, et al. In school-aged children a combination of galacto-oligosaccharides and *Lactobacillus* GG increases bifidobacteria more than *Lactobacillus* GG on its own. Ann Nutr Metab. 2008;52:204–8.
104. Korterink JJ, Ockeloen L, Benninga MA, Tabbers MM, Hilbink M, Deckers-Kocken JM. Probiotics for childhood functional gastrointestinal disorders: a systematic review and meta-analysis. Acta Paediatr. 2014;103:365–72.
105. Yang YJ, Sheu BS. Probiotics-containing yogurts suppress *Helicobacter pylori* load and modify immune response and intestinal microbiota in the *Helicobacter pylori*-infected children. Helicobacter. 2012;17:297–304.
106. Szajewska H, Horvath A, Piwowarczyk A. Meta-analysis: the effects of *Saccharomyces boulardii* supplementation on

Helicobacter pylori eradication rates and side effects during treatment. Aliment Pharmacol Ther. 2010;32:1069–79.
107. Szajewska H, Albrecht P, Topczewska-Cabanek A. Randomized, double-blind, placebo-controlled trial: effect of *Lactobacillus* GG supplementation on *Helicobacter pylori* eradication rates and side effects during treatment in children. J Pediatr Gastroenterol Nutr. 2009;48:431–6.
108. Malfertheiner P, Selgrad M, Bornschein J. *Helicobacter pylori*: clinical management. Curr Opin Gastroenterol. 2012;28:608–14.
109. Wilhelm SM, Johnson JL, Kale-Pradhan PB. Treating bugs with bugs: the role of probiotics as adjunctive therapy for *Helicobacter pylori*. Ann Pharmacother. 2011;45:960–6.
110. Li S, Huang XL, Sui JZ, Chen SY, Xie YT, Deng Y, et al. Meta-analysis of randomized controlled trials on the efficacy of probiotics in *Helicobacter pylori* eradication therapy in children. Eur J Pediatr. 2014;173:153–61.
111. Tabbers MM, de Milliano I, Roseboom MG, Benninga MA. Is *Bifidobacterium breve* effective in the treatment of childhood constipation? Results from a pilot study. Nutr J. 2011;10:19.
112. Bekkali NL, Bongers ME, Van den Berg MM, Liem O, Benninga MA. The role of a probiotics mixture in the treatment of childhood constipation: a pilot study. Nutr J. 2007;6:17.
113. Bu LN, Chang MH, Ni YH, Chen HL, Cheng CC. *Lactobacillus casei* rhamnosus Lcr35 in children with chronic constipation. Pediatr Int. 2007;49:485–90.
114. Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. PLoS One. 2012;7:e34938.
115. Coccorullo P, Strisciuglio C, Martinelli M, Miele E, Greco L, Staiano A. *Lactobacillus reuteri* (DSM 17938) in infants with functional chronic constipation: a double-blind, randomized, placebo-controlled study. J Pediatr. 2010;157:598–602.
116. Guerra PV, Lima LN, Souza TC, Mazochi V, Penna FJ, Silva AM, et al. Pediatric functional constipation treatment with *Bifidobacterium*-containing yogurt: a crossover, double-blind, controlled trial. World J Gastroenterol. 2011;17:3916–21.
117. Tabbers MM, Chmielewska A, Roseboom MG, Crastes N, Perrin C, Reitsma JB, et al. Fermented milk containing *Bifidobacterium lactis* DN-173 010 in childhood constipation: a randomized, double-blind, controlled trial. Pediatrics. 2011;127:e1392–9.
118. Tabbers MM, Boluyt N, Berger MY, Benninga MA. Nonpharmacologic treatments for childhood constipation: systematic review. Pediatrics. 2011;128:753–61.
119. Chmielewska A, Szajewska H. Systematic review of randomised controlled trials: probiotics for functional constipation. World J Gastroenterol. 2010;16:69–75.
120. Hoyos AB. Reduced incidence of necrotizing enterocolitis associated with enteral administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to neonates in an intensive care unit. Int J Infect Dis. 1999;3:197–202.
121. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol Neonate. 2002;82:103–8.
122. Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics. 2005;115:1–4.
123. Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. J Pediatr. 2005;147:192–6.
124. Akisu M, Baka M, Yalaz M, Huseyinov A, Kultursay N. Supplementation with *Saccharomyces boulardii* ameliorates hypoxia/reoxygenation-induced necrotizing enterocolitis in young mice. Eur J Pediatr Surg. 2003;13:319–23.
125. Costalos C, Skouteri V, Gounaris A, Sevastiadou S, Triandafilidou A, Ekonomidou C, et al. Enteral feeding of premature infants with *Saccharomyces boulardii*. Early Hum Dev. 2003;74:89–96.
126. Alfaleh K, Bassler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2008;(1):CD005496.
127. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2011;(3):CD005496.
128. Downard CD, Renaud E, St Peter SD, Abdullah F, Islam S, Saito JM, et al. Treatment of necrotizing enterocolitis: an American Pediatric Surgical Association Outcomes and Clinical Trials Committee systematic review. J Pediatr Surg. 2012;47:2111–22.
129. Mihatsch WA, Braegger CP, Decsi T, Kolacek S, Lanzinger H, Mayer B, et al. Critical systematic review of the level of evidence for routine use of probiotics for reduction of mortality and prevention of necrotizing enterocolitis and sepsis in preterm infants. Clin Nutr. 2012;31:6–15.
130. Fallon EM, Nehra D, Potemkin AK, Gura KM, Simpser E, Compher C, et al. A.S.P.E.N. clinical guidelines: nutrition support of neonatal patients at risk for necrotizing enterocolitis. JPEN J Parenter Enteral Nutr. 2012;36:506–23.
131. Janvier A, Lantos J, Barrington K. The politics of probiotics: probiotics, necrotizing enterocolitis and the ethics of neonatal research. Acta Paediatr. 2013;102:116–8.
132. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2014;4:CD005496.
133. Savino F, Pelle E, Palumeri E, Oggero R, Miniero R. *Lactobacillus reuteri* (American type culture collection strain 55730) versus simethicone in the treatment of infantile colic: a prospective randomized study. Pediatrics. 2007;119:e124–30.
134. Savino F, Cordisco L, Tarasco V, Palumeri E, Calabrese R, Oggero R, et al. *Lactobacillus reuteri* DSM 17938 in infantile colic: a randomized, double-blind, placebo-controlled trial. Pediatrics. 2010;126:e526–33.
135. Szajewska H, Gyrczuk E, Horvath A. *Lactobacillus reuteri* DSM 17938 for the management of infantile colic in breastfed infants: a randomized, double-blind, placebo-controlled trial. J Pediatr. 2013;162:257–62.
136. Sung V, Hiscock H, Tang ML, Mensah FK, Nation ML, Satzke C, et al. Treating infant colic with the probiotic *Lactobacillus reuteri*: double blind, placebo controlled randomised trial. BMJ. 2014;348:g2107.
137. Dupont C, Rivero M, Grillon C, Belaroussi N, Kalindjian A, Marin V. Alpha-lactalbumin-enriched and probiotic-supplemented infant formula in infants with colic: growth and gastrointestinal tolerance. Eur J Clin Nutr. 2010;64:765–7.
138. Kianifar H, Ahanchian H, Grover Z, Jafari S, Noorbakhsh Z, Khakshour A, et al. Synbiotic in the management of infantile colic: a randomised controlled trial. J Paediatr Child Health. 2014 Jun 24 [Epub ahead of print].
139. Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, et al. Long-term safety and impact on infection rates of postnatal probiotic and prebiotic

(synbiotic) treatment: randomized, double-blind, placebo-controlled trial. Pediatrics. 2008;122:8–12.
140. Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. J Allergy Clin Immunol. 2007;119:184–91.
141. Osborn DA, Sinn JK. Probiotics in infants for prevention of allergic disease and food hypersensitivity. Cochrane Database Syst Rev. 2007;(4):CD006475.
142. Dotterud CK, Storrø O, Johnsen R, Oien T. Probiotics in pregnant women to prevent allergic disease: a randomized, double-blind trial. Br J Dermatol. 2010;163:616–23.
143. Doege K, Grajecki D, Zyriax BC, Detinkina E, Zu Eulenburg C, Buhling KJ. Impact of maternal supplementation with probiotics during pregnancy on atopic eczema in childhood-a meta-analysis. Br J Nutr. 2012;107:1–6.
144. Wickens K, Black P, Stanley TV, Mitchell E, Barthow C, Fitzharris P, et al. A protective effect of *Lactobacillus rhamnosus* HN001 against eczema in the first 2 years of life persists to age 4 years. Clin Exp Allergy. 2012;42:1071–9.
145. Pelucchi C, Chatenoud L, Turati F, Galeone C, Moja L, Bach JF, et al. Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: a meta-analysis. Epidemiology. 2012;23:402–14.
146. van der Aa LB, van Aalderen WM, Heymans HS, Henk Sillevis Smitt J, Nauta AJ, Knippels LM, et al. Synbiotics prevent asthma-like symptoms in infants with atopic dermatitis. Allergy. 2011;66:170–7.
147. Bath-Hextall FJ, Jenkinson C, Humphreys R, Williams HC. Dietary supplements for established atopic eczema. Cochrane Database Syst Rev. 2012;2:CD005205.
148. Wu KG, Li TH, Peng HJ. *Lactobacillus salivarius* plus fructo-oligosaccharide is superior to fructo-oligosaccharide alone for treating children with moderate to severe atopic dermatitis: a double-blind, randomized, clinical trial of efficacy and safety. Br J Dermatol. 2012;166:129–36.
149. Han Y, Kim B, Ban J, Lee J, Kim BJ, Choi BS, et al. A randomized trial of *Lactobacillus plantarum* CJLP133 for the treatment of atopic dermatitis. Pediatr Allergy Immunol. 2012;23:667–73.
150. Gore C, Custovic A, Tannock GW, Munro K, Kerry G, Johnson K, et al. Treatment and secondary prevention effects of the probiotics *Lactobacillus paracasei* or *Bifidobacterium lactis* on early infant eczema: randomized controlled trial with follow-up until age 3 years. Clin Exp Allergy. 2012;42:112–22.
151. Thomas DW, Greer FR. American Academy of Pediatrics Committee on Nutrition; American Academy of Pediatrics Section on Gastroenterology Hepatology, and Nutrition. Probiotics and prebiotics in pediatrics Pediatrics. 2010;126:1217–31.
152. Kim SO, Ah YM, Yu YM, Choi KH, Shin WG, Lee JY. Effects of probiotics for the treatment of atopic dermatitis: a meta-analysis of randomized controlled trials. Ann Allergy Asthma Immunol. 2014;113:217–26.
153. van der Aa LB, Lutter R, Heymans HS, Smids BS, Dekker T, van Aalderen WM, et al. No detectable beneficial systemic immunomodulatory effects of a specific synbiotic mixture in infants with atopic dermatitis. Clin Exp Allergy. 2012;42:531–9.
154. Sazawal S, Dhingra U, Hiremath G, Sarkar A, Dhingra P, Dutta A, et al. Prebiotic and probiotic fortified milk in prevention of morbidities among children: community-based, randomized, double-blind, controlled trial. PLoS One. 2010;5:e12164.
155. Kumar S, Singhi S. Role of probiotics in prevention of *Candida* infection in critically ill children. Mycoses. 2013;56:204–11.
156. Sanz-Penella JM, Frontela C, Ros G, Martinez C, Monedero V, Haros M. Application of bifidobacterial phytases in infant cereals: effect on phytate contents and mineral dialyzability. J Agric Food Chem. 2012;60:11787–92.
157. Capozzi V, Russo P, Dueñas MT, López P, Spano G. Lactic acid bacteria producing B-group vitamins: a great potential for functional cereals products. Appl Microbiol Biotechnol. 2012;96:1383–94.
158. Kadooka Y, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, et al. Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr. 2010;64:636–43.
159. Andreasen AS, Larsen N, Pedersen-Skovsgaard T, Berg RM, Møller K, Svendsen KD, et al. Effects of *Lactobacillus acidophilus* NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. Br J Nutr. 2010;104:1831–8.
160. Luoto R, Kalliomäki M, Laitinen K, Isolauri E. The impact of perinatal probiotic intervention on the development of overweight and obesity: follow-up study from birth to 10 years. Int J Obes (Lond). 2010;34:1531–7.
161. Lo Vecchio A, Cohen MB. Fecal microbiota transplantation for *Clostridium difficile* infection: benefits and barriers. Curr Opin Gastroenterol. 2014;30:47–53.
162. Rubin DT. Curbing our enthusiasm for fecal transplantation in ulcerative colitis. Am J Gastroenterol. 2013;108:1631–3.
163. Kunde S, Pham A, Bonczyk S, Crumb T, Duba M, Conrad H Jr, et al. Safety, tolerability, and clinical response after fecal transplantation in children and young adults with ulcerative colitis. J Pediatr Gastroenterol Nutr. 2013;56:597–601.
164. Vrieze A, de Groot PF, Kootte RS, Knaapen M, van Nood E, Nieuwdorp M. Fecal transplant: a safe and sustainable clinical therapy for restoring intestinal microbial balance in human disease? Best Pract Res Clin Gastroenterol. 2013;27:127–37.
165. Quera R, Espinoza R, Estay C, Rivera D. Bacteremia as an adverse event of fecal microbiota transplantation in a patient with Crohn's disease and recurrent *Clostridium difficile* infection. J Crohns Colitis. 2014;8:252–3.
166. Vandenplas Y, Veereman G, van der Werff Ten Bosch J, Goossens A, Pierard D, Samsom JN, et al. Fecal microbial transplantation in a one-year-old girl with early onset colitis - caution advised. J Pediatr Gastroenterol Nutr. 2014 Jan 2 [Epub ahead of print].
167. Shanahan F. A commentary on the safety of probiotics. Gastroenterol Clin North Am. 2012;41:869–76.
168. Fedorak RN, Madsen KL. Probiotics and prebiotics in gastrointestinal disorders. Curr Opin Gastroenterol. 2004;20:146–55.
169. Allen SJ, Jordan S, Storey M, Thornton CA, Gravenor M, Garaiova I, et al. Dietary supplementation with lactobacilli and bifidobacteria is well tolerated and not associated with adverse events during late pregnancy and early infancy. J Nutr. 2010;140:483–8.
170. Martín-Muñoz MF, Fortuni M, Caminoa M, Belver T, Quirce S, Caballero T. Anaphylactic reaction to probiotics. Cow's milk and hen's egg allergens in probiotic compounds Pediatr Allergy Immunol. 2012;23:778–84.
171. Borriello SP, Hammes WP, Holzapfel W, Marteau P, Schrezenmeir J, Vaara M, et al. Safety of probiotics that contain lactobacilli or bifidobacteria. Clin Infect Dis. 2003;36:775–80.

172. Mackay AD, Taylor MB, Kibbler CC, Hamilton-Miller JM. *Lactobacillus* endocarditis caused by a probiotic organism. Clin Microbiol Infect. 1999;5:290–2.
173. Rautio M, Jousimies-Somer H, Kauma H, Pietarinen I, Saxelin M, Tynkkynen S, et al. Liver abscess due to a *Lactobacillus rhamnosus* strain indistinguishable from *L. rhamnosus* strain GG. Clin Infect Dis. 1999;28:1159–60.
174. Cabana MD, Shane AL, Chao C, Oliva-Hemker M. Probiotics in primary care pediatrics. Clin Pediatr (Phila). 2006;45:405–10.
175. Dai M, Lu J, Wang Y, Liu Z, Yuan Z. *In vitro* development and transfer of resistance to chlortetracycline in *Bacillus subtilis*. J Microbiol. 2012;50:807–12.