Probiotics in gastrointestinal-associated diseases

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Disturbance of the bacterial microflora of the gastrointestinal (GI) tract has been associated with the pathophysiology of several GI disorders. Manipulation of the gut microbiota through supplementation with probiotics is an attractive approach to restore, maintain and promote health. Common probiotic formulations for human consumption are mainly based on bacterial strains of *Lactobacillus*, *Bifidobacterium* and the yeast *Saccharomyces boulardii*, either as single or multispecies preparations. Clinical efficacy of a probiotic product is determined by the specific microbial strain, formulation, dose regimen, viability of the microorganisms and time of permanence in the gut. Evidence from research studies and clinical data support the use of probiotics in the prevention and/or treatment of several GI disorders caused either by perturbations in normal gut microbiota, such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), or by pathogenic microorganisms, including GI infections caused by *Helicobacter pylori*, *Clostridium difficile*, and rotavirus. This review discusses the role of probiotics in the prevention and treatment of GI disorders, and proposed mechanisms of action.

Keywords: probiotics; gastrointestinal disorders; inflammatory bowel disease; irritable bowel syndrome; *Helicobacter pylori*; *Clostridium difficile*

1. Introduction

Gastrointestinal (GI) disorders, such as chronic inflammatory GI conditions (e.g., Crohn's disease, ulcerative colitis, irritable bowel syndrome) and acute GI infections are still a major cause of morbidity and mortality worldwide. Many of these diseases, particularly those requiring antibiotic therapy, are becoming increasingly difficult to treat due to increased dissemination of antibiotic resistance among microorganisms and the emergence of (multi)drug-resistant strains [1]. Novel therapeutic approaches are thus essential, and manipulation of the gut microbiota through supplementation with probiotics is an attractive alternative to conventional therapies. Moreover, probiotics are amenable to low-cost and large scale production.

Probiotics have been used in the treatment and prevention of many GI-associated diseases, either alone or as co-adjuvants of standard therapies, with promising results. Probiotic beneficial effects are likely the result of multiple mechanisms of action in the gut that may include effects on host immunity and gut mucosal barrier integrity as well as colonization resistance and production of antimicrobial substances [2]. While some of these mechanisms may be shared among different strains or species, others are specific to certain microorganisms.

Probiotics are associated with a good safety record in the healthy population, primarily related to the use of lactobacilli and bifidobacteria. On the other hand, selection and monitoring of probiotics for individuals compromised gut epithelial integrity and immunocompromised patients demands careful consideration of the risk-benefit ratio.

Translational to the clinical setting requires specification of effective strains or strain combinations and dosage regimen, since therapeutic effects of probiotics are both strain- and dose-dependent [2,3].

2. Mechanisms of Action

Several mechanisms of action are ascribed to probiotics, corresponding to genetic and functional differences in the bacterial strains (even within the same species) [4-6]. A good example is the *Lactobacillus reuteri* strains, present in formulations of probiotics for intestinal applications. While *L. reuteri* SD2112 (ATCC55730) produce the antimicrobial metabolite reuterin that inhibits pathogens in the gut, *L. reuteri* RC-14 health benefits on the intestine are associated to the production of biosurfactants and other antiadhesive constituents as well as to modulating host immunity [4]. Conversely, the same strain might exert its biological effects by multiple mechanisms of action [6]; an example is *L. rhamnosus* GG (LGG) that could reduce inflammation and concomitantly induce adaptive immune cells. Some probiotic strains are capable of producing effects far away from the site of administration. Reasons may lie in the transfer of the organisms from one site to another or in the production of molecules that are adsorbed through the intestine [6]. In general, it is possible to identify a variety of potential mechanisms of action through which probiotics exert their effect on GI-associated disorders [6–15]. Some of the proposed mechanisms, supported by *in vitro* and some *in vivo* experiments, are briefly described.

2.1 Modulation of the host immune system

The intestinal microbiota is fundamental for the maturation of the host immune system and development of immunological resistance. Probiotic bacteria or their metabolites have the capacity to interact with epithelial cells and

immune cells through pattern recognition receptors [5,7,9–12,14,16,17]. Two of these highly specific receptors are Toll-like (microbial-sensing) and NOD (intracellular-sensing) proteins. Toll-like receptors signaling stimulates expression of defensins (small peptides/proteins) in enterocytes [18]. After recognition, immune and epithelial cells produce cytokines that contribute to the control of innate and adaptive immune cells. Lymphocytes (B and T cells) are essential pieces in the adaptive immune response. Many probiotics seem to induce the activation of T helper cells, thus producing cytokines and interfering with immune responses [10].

Immunomodulation by probiotics comprises also the increase of immunoglobulin A (IgA) production by B cells, disabling the proinflammatory response [9,10,14]. Nevertheless, conclusions based on the effect of probiotics on IgA production are still questionable, as some results seem to be contradictory [14]. Although *L. reuteri* led to an increase in proinflammatory cytokines *in vitro*, the same strain revealed an anti-inflammatory activity *in vivo* [7,10].

The immunomodulatory process depends on probiotics survival and permanence in the GI tract, the type of interaction between probiotic and the host immune system, and the strain, dosage and frequency of administration. Immunostimulation of the host seems to be only achieved with a dose of 10^8–10^9 colony forming units (CFU)/d of a strain and a resident time in the intestine between 48 and 72 hours [14].

2.2 Adhesion to intestinal mucosa

Adhesion to intestinal mucosa is the precondition to colonization, being determinant for the probiotic-host interaction, modulation of the immune system, and antagonism against pathogens [9,17].

Intestinal epithelia cells produce the glycoprotein mucin which is the main component of mucus, precluding adhesion of pathogens. Various *Lactobacillus* spp. are reported to adhere to the mucosa, due to mucus adhesion-promoting proteins [5,9,17]. These circumstances might explain an eventual relation between probiotic surface proteins and the concurrent pathogens exclusion from the mucus [9,17]. However, studies on the adhesion-related factors are scarce.

Competitive exclusion of pathogens (where one species of bacteria inhibits or reduces the growth of another species) lies on the competition for nutrients and for mucosal adhesion receptors, being a consequence of the different mechanisms and properties of probiotics [9,14].

2.3 Production of antimicrobial compounds

Considering some GI-related disorders, several authors suggest that probiotics have an impact on microbiota composition positively modifying its qualitative and quantitative profile [14].

Some probiotics can produce antimicrobial compounds which inhibit the growth of bacteria existing in the intestinal lumen [14,17,18]. Lactic and acetic acids have been reported as major antimicrobial compounds of probiotics, due to their high inhibitory activity against Gram-negative bacteria [9,14,17]. The effect on the pathogenic microorganisms may be related to the decrease of intracellular pH or to the intracellular accumulation of the organic acid. Probiotics such as *L. acidophilus* produce lactic acid that, additionally, hamper the adhesion of pathogenic bacteria to epithelial cells and stimulate the clonal expansion of mucosal B lymphocytes to produce IgA [9,14,17].

Along with the aforementioned reuterin (produced by *L. reuteri* SD2112), probiotics may also produce bacteriocins, which are ribosomally synthesized antimicrobial peptides or proteins produced by bacteria, that inhibit the growth of other bacteria reducing the number of pathogens or changing the composition of intestinal microbiota [14,18]. The destruction of pathogen cells results from pore formation and/or inhibition of cell wall synthesis [9]. Some bacteriocins generated by probiotics have been identified. An effective inhibition of foodborne pathogen *Listeria monocytogenes* in mice and pigs was observed for the bacteriocin Abp118, produced by *L. salivarius* UCC118. *Bifidobacterium bifidum* NCFB 1454 produces bifidocin B which inhibits the growth of Gram-positive bacteria [9]. Moreover, probiotic strains can also promote the discharge of defensins by the epithelial cells. Defensins bind to anionic phospholipid groups of the membrane surface through electrostatic interactions, causing the lysis of microorganism cells [7,9,16,18].

Probiotics have also the capacity to deconjugate bile acids [5,6,9,19]. Deconjugated bile acids seems to have a higher antibacterial activity when compared with the ones produced by the host [9].

Several probiotic strains produce metabolites with antifungal properties. In this context, *Lactobacillus* species produce benzoic acid, methylhydantoin, mevalonolactone and short-chain fatty acids (SCFA) [9,17].

2.4 Changes in composition and metabolic activity of gut microbiome

Gut microbiome act as a source of secondary metabolites that are pharmacologically active and able to activate the mammalian liver enzymes [5]. SCFA results from the metabolic activity of intestinal microbiota, and their production seems to be increased by probiotics, which enhances pathogen resistance and stimulates epithelial cells [5,9,14,20]. SCFA have anti-inflammatory properties, being able to enhance epithelial barrier integrity. Production of SCFA also depends on the composition of intestinal microbiota, the diet and the presence of other metabolites [14]. Butyric acid is the most well documented SCFA for colonic epithelial cells, in terms of anti-inflammatory effects [14,21]. Its impact on intestinal function may lie in the inhibition of the activation of nuclear factor kappa B (a prototypical proinflammatory

signaling pathway), in the increased secretion of mucins and antimicrobial peptides, and in the increase of expression of tight junctions proteins [14,20,21].

2.5 Improvement of the intestinal epithelial barrier

Intestinal epithelial cells constitute the surface where immune responses are initiated. A balanced homeostasis of intestinal mucosal function strongly depends on the integrity of the epithelial cells barrier between the intestinal lumen, the lamina propria and the mucosal-associated lymphoid tissue. Microorganisms composing the intestinal microbiota may change the intestinal barrier, making it more or less permeable [14,18]. An increase in mucosal permeability and loss of epithelial integrity are relevant pathophysiological manifestations when considering the GI disorders under analysis. Some species of probiotics show the capacity to diminish the intestinal permeability due to changes in the intracolonic pH, the cellular junction proteins that form the tight junctions and promote colonocytes self-adhesion, and probably in the production of mucins [7,18]. Moreover, defensins also stabilize the gut barrier function [7,16].

3. Therapeutic applications

3.1 Diarrhea treatment and prevention

3.1.1 Treatment of acute infectious diarrhea

Acute infectious diarrhea is a leading cause of mortality worldwide in infants and hospitalized children, especially in developing countries [10]. Most cases of acute, watery diarrhea are due to viral gastroenteritis, which in children are usually caused by rotavirus. On the other hand, traveler's diarrhea and foodborne diarrheas are commonly of bacterial origin (e.g., enterotoxigenic *Escherichia coli* (ETEC), *Campylobacter*, *Shigella*, or *Salmonella*) [22].

Probiotics have been extensively studied in the treatment and prevention of acute diarrheal states, especially in the pediatric population. Most of the studies involved lactobacilli strains (mainly *Lactobacillus rhamnosus* GG, *L. reuteri*, *L. casei* and *L. acidophilus*), *Bifidobacterium lactis*, *Streptococcus thermophilus* and the yeast *Saccharomyces boulardii*, used either alone or in combination [2,23].

Data from several placebo-controlled, randomized clinical trials (RCTs) point to a statistically significant benefit in the use of probiotics, mostly LGG and *S. boulardii*, for the treatment of acute infectious diarrhea [2,3,19,23–27]. The effect of probiotics is both strain-dependent and dose-dependent, with doses higher than 10^{10}–10^{11} CFU/d usually achieving better results [23,24,28]. Fang et al. [29] studied the dose-dependent effect of *L. casei rhamnosus* Lcr35 in children with acute rotaviral gastroenteritis and found that a minimal effective dose of 6×10^{8} CFU daily, for 3 days, was necessary for quantitative reduction of fecal rotavirus shedding in pediatric patients [29].

Probiotics seem to be more effective in the treatment of acute watery diarrhea, particularly if caused by rotavirus, than in invasive bacterial diarrhea, and achieve better therapeutic effect when administered early in the course of the diarrheal state [23,24]. The efficacy of a probiotic formulation combining *Bacillus mesentericus*, *Clostridium butyricum* and *Enterococcus faecalis* in the treatment of acute infectious diarrhea in hospitalized children with *Salmonella* and rotavirus gastroenteritis revealed significant reduction in the severity of symptoms for rotavirus-infected children only [30].

The available body of evidence supports recent guidelines from both the European Society for Pediatric Infectious Diseases (ESPID) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) that recommend specific probiotics with proven clinical efficacy, namely LGG and *S. boulardii* CNCM I-745, as an adjunct to rehydration therapy for the management of acute gastroenteritis in children [31].

Probiotics have been found to shorten the duration of acute infectious diarrhea by approximately 1 day in adults and children, and reduce stool frequency on day 2, according to a Cochrane review involving 63 studies with a total of more than 8,000 participants, mainly receiving LGG, *S. boulardii* or *Enterococcus faecium* SF68 [2]. However, *E. faecium* SF68 is a possible recipient of the vancomycin-resistance genes and it is not advisable in the management of children with acute gastroenteritis [2]. The probiotic formulation VSL#3, a mixture of 8 probiotic strains (*L. acidophilus*, *L. paracasei*, *L. bulgaricus*, *L. plantarum*, *B. breve*, *B. infantis*, *B. longum* and *S. thermophilus*) is preferable for the pediatric treatment of acute rotavirus diarrhea. Oral administration of VSL#3 during 4 days to infected children significantly reduced stool frequency and the requirement for oral rehydration salts compared to the placebo without showing side effects, resulting in better recovery rates [1,32].

3.1.2 Prevention of acute infectious diarrhea

There is evidence that certain probiotics can prevent acute diarrheal states, both in adult and children populations. A meta-analysis of 34 placebo-controlled, RCTs evaluating the efficacy of probiotics in preventing acute diarrhea revealed a reduction of 35% with substantial heterogeneity [33]. The effect was found to depend on the probiotic strain and on the age of the host, with probiotics reducing the risk of acute diarrhea in children by 57% but only by 26% in adults [33].

In a double-blind RCT, oral administration of LGG (6×10^9 CFU/twice daily) to 81 children hospitalized for non-diarrheal complaints significantly reduced the risk of nosocomial diarrhea in comparison with placebo, particularly nosocomial rotaviral gastroenteritis [27,34]. In another study, supplementation of an infant formula with *B. bifidum* and *S. thermophilus* reduced the incidence of acute diarrhea and rotavirus shedding in infants admitted to hospital [10,27].

On the other hand, administration of *L. reuteri* DSM 17938 did not significantly reduce the risk of nosocomial diarrhea or rotavirus gastroenteritis in hospitalized children [35]. However, daily administration of *L. reuteri* DSM 17938 (10^8 CFU/d) for 3 months to healthy children attending day care centers reduced the frequency and duration of diarrheal episodes compared to placebo [36]. Moreover, a study conducted in a community setting indicates that daily intake of a probiotic drink containing *L. casei* strain Shirota for 12 weeks reduced the occurrence of acute diarrhea in young children compared to a non-supplemented nutrient drink [37].

Regarding the prophylaxis of traveler's diarrhea (TD), which is a common health problem when travelling to Africa, South East Asia, or Central and South America, a meta-analysis of 12 RCTs by McFarland [22] concluded that probiotics, namely *S. boulardii* and a mixture of *L. acidophilus* and *B. bifidum*, are safe and effective for this purpose [22]. Probiotic use was associated with a significant reduced risk of 85% in developing TD [38]. The efficacy of the treatment depended on duration, dosage and type of probiotic, traveler compliance, travel destination and probiotic viability during the trip. Further studies are needed before probiotics can be considered as an alternative option to antibiotic therapy for the prophylaxis of TD.

3.1.3 Prevention of antibiotic-associated diarrhea

Diarrhea is often a side effect of antibiotic therapy due to disturbance of the normal host GI microflora enhancing overgrowth of enteropathogens, such as *Clostridium difficile*, responsible for diarrhea and colonic inflammation (colitis) [1]. Prevention of antibiotic-associated diarrhea (AAD) by probiotics may be mediated by several mechanisms, such as local competition for adhesion receptors and nutrients, production of antimicrobial substances, and stimulation of intestinal antigen and nonspecific immune responses that enhance restoration of the gut microflora [39].

One third of the patients on antibiotic therapy commonly develop AAD at any point from the initiation of the treatment up to several weeks after its discontinuation [39,40]. Several factors can influence the development of AAD, such as the type of antibiotic, age and health conditions of the host, etiology and hospitalization status. A higher risk of AAD is commonly linked to antibiotics that act on anaerobes, including broad-spectrum penicillins, cephalosporins, clindamycin and fluoroquinolones [41–43].

Several meta-analysis and systematic reviews indicate a statistically significant association between probiotic administration and reduction of AAD, with the most effective strains being LGG and *S. boulardii* [23,38–42]. These probiotics are actually recommended for the prevention of AAD in children by the ESPGHAN working group on probiotics [43].

L. reuteri ATCC 55730 has also been shown to reduce the incidence of AAD in adults in a hospital setting [44], however this strain carries transferable resistance traits for tetracycline and lincomycin, and should preferably be replaced by *L. reuteri* DSM 17938, with no unwanted plasmid-borne resistances [26]. A RCT to assess the efficacy of *L. reuteri* DSM 17938 for the prevention of diarrhea and AAD in children (NCT02871908) is currently recruiting [45].

Since the beneficial effects of probiotics are strain-specific, some meta-analysis have been performed on the efficacy and safety of only one type of microorganism in order to avoid heterogeneity from pooling data on different strains [38,41,42]. A systematic review with meta-analysis of *S. boulardii* for the prevention of AAD in children and adults showed that 1 in 10 cases of AAD could be prevented by co-administration of the probiotic [41].

The dose-response effect of probiotics on the incidence of AAD has also been evaluated. In hospitalized adult patients on antibiotic therapy, daily administration of a probiotic mixture (*L. acidophilus* NCFM, *L. paracasei* Lpc-37, *B. lactis* Bi-07 and Bi-04) up to 7 days after the end of the antibiotic course showed a significant dose-response effect on AAD, with incidences of 12.5% in patients on the high-dose regimen (1.70×10^{10} CFU/d) compared with 19.5% in the low-dose regimen (4.17×10^9 CFU/d) group and 24.6% in the placebo group [46].

It is worth mention that in most of these studies, the type and/or dose of the antibiotic have not been randomized along with the probiotic arm. In healthy volunteers taking amoxicillin (500 mg twice daily for 7 days) and 5 g of a multispecies probiotic preparation (10^9 CFU/g twice daily for 15 days) containing *B. bifidum* W23, *B. lactis* W18, *B. longum* W51, *E. faecium* W54, *L. acidophilus* W37 and W55, *L. paracasei* W72, *L. plantarum* W62, *L. rhamnosus* W71, and *L. salivarius* W24, changes in microbial composition and metabolic activity of the intestinal microbiota over time suggested that the amoxicillin effect was modulated by probiotic intake [39].

3.1.4 *Clostridium difficile*-associated diarrhea

Clostridium difficile infection (CDI) is a common complication of antibiotic therapy, with symptoms ranging from mild diarrhea and colonic inflammation to severe pseudomembranous enterocolitis, which can lead to sepsis and death [47,48]. *Clostridium difficile*-associated diarrhea (CDAD) is most frequent in elderly and hospitalized patients receiving broad-spectrum antibiotics (e.g., penicillins, cephalosporins, clindamycin and fluoroquinolones) but other risk factors include the use of proton-pump inhibitors, H_2-antagonists, methotrexate, and existence of other GI pathologies

associated with impaired mucosal barrier function [49]. Metronidazole and vancomycin are the standard therapeutic regimens for CDI, however recurrence and relapse have been observed, even after repeated antibiotic treatments [47, 49].

Probiotics can prevent gut colonization by *C. difficile* and are thus promising agents for the prophylaxis of CDAD. Moreover, certain strains of lactobacilli produce peptide metabolites with lytic activity against several *C. difficile* strains while *S. boulardii* produces a serine protease that degrades *C. difficile* toxins A and B [38,50]. Co-treatment with probiotic *S. boulardi* is associated with a significant decrease in the risk of CDI recurrence [38,41].

Fecal sample analysis of *C. difficile* toxins in hospitalized elderly patients on antibiotic therapy revealed that 46% of the patients who received co-treatment with a probiotic mixture containing *L. acidophilus* and *B. bifidum* were toxin-positive compared with 78% in the placebo group [47–49]. Consumption of cheese supplemented with both *L. rhamnosus* HN001 and *L. acidophilus* NCFM by healthy, elderly volunteers was also associated with a trend towards lower fecal counts of *C. difficile* compared with the non-supplemented cheese for the same period [51].

Co-administration of a probiotic drink containing *L. casei*, *L. bulgaricus* and *S. thermophilus* to hospitalized elderly patients (mean age 74 years old) on antibiotic therapy lowered the incidence of CDAD [47–49]. Moreover, a dose-response efficacy study with a probiotic formulation containing *L. acidophilus* CL1285 and *L. casei* LBC80R (50 billion CFU/capsule) for CDAD prophylaxis showed that high doses (2 capsules/d) were more efficient than low doses (1 capsule/d) in reducing the incidence of CDAD in hospitalized adult patients [47–49,52].

Contradictory results have been reported on the efficacy of probiotics for the prevention of recurrent CDI, which may be due to differences in the study population, type and dose of probiotic, and/or duration of the treatment [53]. A recent meta-analysis and a Cochrane review have found that probiotics significantly reduce the risk of CDAD by approximately 60% [48,49] but not the incidence of CDI [48].

On the other hand, lower CDI rates have been found among antibiotic-treated patients taking probiotics during time at risk when compared with placebo, and results from a meta-analysis suggest that primary prevention of CDI can be achieved with specific probiotic agents, the most promising ones being *S. boulardii* and the probiotic mixture *L. acidophilus* CL1285 and *L. casei* LBC80R [53]. A probiotic combination of *L. acidophilus* CL1285, *L. casei* LBC80R, and *L. rhamnosus* CLR2 containing 50 billion live bacteria, commercially available as both fermented beverages and targeted-release capsules with an enteric coating, has been associated with decreased incidences of CDI in human clinical trials [54]. The commercial products, as well as the individual strains, have shown antimicrobial activity against *C. difficile* and ability to neutralize toxins A and B *in vitro* [54].

Overall, evidence from systematic reviews and meta-analysis suggest a beneficial effect of probiotics as adjuvant therapy in preventing primary CDI, but there is insufficient data to support their use in secondary prevention of recurrent CDI, requiring larger studies for further evaluation [53,54]. Moreover, antibiotic regimens need to be specified in order to determine the influence of antibiotic class or type, dose and duration of treatment in probiotic efficacy [53,55].

3.2 Helicobacter pylori eradication

Helicobacter pylori are a group of highly prevalent human pathogens infecting approximately half of the world population [56]. *H. pylori* infection has been linked to dyspepsia, chronic gastritis, peptic ulcer, and gastric cancer [56]. Current treatment guidelines recommend concomitant triple therapy for 7-14 days combining a proton pump inhibitor (PPI) with the antibiotics clarithromycin and either amoxicillin or metronidazole as the first approach for *H. pylori* eradication [56,57]. However, eradication rates have declined from 90% to 70% with this treatment, mainly due to increase in antibiotic resistance and poor patient compliance resulting from frequent adverse effects associated with antibiotic therapy, such as diarrhea, headache, loss of appetite, nausea, abdominal pain and skin rash [56,57].

In order to overcome antibiotic resistance, levofloxacin-containing triple therapy as well as sequential and concomitant quadruple therapies with addition of either a bismuth salt or another antibiotic have been developed as second line treatments for *H. pylori* eradication, with quadruple regimens being associated with higher incidence of adverse side effects [56,57].

Probiotics can prevent GI-related side effects of antibiotic therapy, particularly AAD, by aiding in the restoration of gut microbiota. Moreover, probiotics such as *Lactobacillus*, *Bifidobacterium* and *S. boulardii* have demonstrated anti-*H. pylori* activity both *in vitro* and in animal models of *H. pylori* infection [17,56].

The *H. pylori* inhibitory effect of probiotics may be due to competition with the pathogen for adhesion sites, production of antimicrobial metabolites (e.g., *L. johnsonii* La1, *L. casei* strain Shirota, *L. lactis*, *Bacillus subtilis*) or by enhancing mucin secretion (e.g., *L. plantarum* 299v and LGG). Lactic acid production by lactic acid bacteria (LAB) and bifidobacteria decreases pH, which attenuates the hypochlorhydria associated with *H. pylori* infection and can also inhibit *H. pylori* urease [17,56]. Moreover, probiotics show immunomodulatory effects that reduce *H. pylori*-induced gastric activity and inflammation by controlling the balance of proinflammatory and anti-inflammatory cytokines [17,56]. A decrease in specific IgG antibodies to *H. pylori* infection parallel to a reduction of gastric inflammation has been observed in animal models following probiotic intake, with simultaneous enhancement of secretory IgA production in the intestinal epithelium and strengthening of the mucosal barrier [17,56].

Probiotic monotherapy using strains such as *L. johnsonii* La1, *L. casei* strain Shirota, *L. gasseri* OLL2716, *L. reuteri* ATCC 55730, *L. acidophilus* La5 or *B. bifidum* BF-1 has been shown to decrease bacterial load in humans, but probiotics alone are not able to eradicate *H. pylori* [17,57]. On the other hand, several systematic reviews and meta-analysis suggest that adjuvant therapy with probiotic *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, and their blends, can significantly increase eradication rates in comparison with placebo or no additional treatment while simultaneous reducing antibiotic-related side effects (particularly AAD) and improving patient compliance [41,56,58–60]. Sub-group analysis concerning data from 33 RCTs involving a total of 4,459 patients showed that the impact of probiotic supplementation on the efficacy of *H. pylori* eradication therapy was statistically significant only for some individual strains: *L. acidophilus*, *L. casei* DN-114001, *L. gasseri*, and *B. infantis* 20136 [58].

H. pylori eradication rates improved from 68.9% to 83% after addition of the probiotic *B. infantis* 2036 to standard triple therapy [56,58]. On the other hand, probiotic pretreatment for 2 weeks before addition to either triple therapy or sequential therapy increased eradication rates up to 90% [56,58]. Furthermore, a 4-week treatment with *L. gasseri* OLL2716-containing yogurt twice daily 3 weeks prior first-line *H. pylori* eradication therapy, improved eradication rate of primary clarithromycin-resistant *H. pylori* strains (compared to triple therapy alone), in agreement with previous *in vitro* studies showing the ability of *L. gasseri* OLL2716 to suppress both clarithromycin-susceptible and -resistant *H. pylori* strains [56,58].

A 2-week supplementation with 100 mL of fermented milk containing probiotic *L. casei* DN-114 001 enhanced the therapeutic benefit of standard triple therapy in *H. pylori*-positive children with gastritis [56,58]. Kefir, a fermented milk drink containing *Lactobacillus*, *Lactococcus* and yeast, has also been found to increase eradication rate in dyspepsia patients on standard triple therapy when compared with placebo-containing milk [56,58].

Other studies support the beneficial effect of *L. reuteri* strains (known to secrete the antimicrobial metabolite reuterin that inhibits *H. pylori* growth *in vitro*), which have been associated with increased eradication rates, decreased serum gastrin-17 levels in *H. pylori* asymptomatic patients, and decreased incidence of adverse events both in first-line triple therapy and in second-line triple therapy with levofloxacin [56,58].

Regarding probiotic blends as adjuvants in *H. pylori* eradication therapy, a recent systematic review and meta-analysis [60] indicate that the most effective multi-strain probiotics are a combination of *L. acidophilus* and *B. animalis*, and an eight-strain mixture composed of *L. acidophilus*, *L. casei rhamnosus*, *L. plantarum*, *L. reuteri*, *L. salivarius*, *L. sporogenes*, *B. infantis*, and *B. longum* [60].

Overall, the benefits conferred by probiotics in coadjuvant therapy for *H. pylori* eradication are strain-specific and influenced by the chosen antibiotic regimen, being more significant with relatively ineffective antibiotic regimens [56,58,59].

3.3 Inflammatory bowel disease

Inflammatory bowel disease (IBD) comprises idiopathic chronic relapsing inflammatory disorders of the GI tract, such as Crohn's disease (CD) and ulcerative colitis (UC). In the former, inflammation is often discontinuous, transmural and may involve any part of the GI tract (although predominantly affecting the terminal ileum and colon) while in the latter inflammation is typically continuous with mucosal lesions confined to the colon [18,61]. The pathophysiology of IBD is multifactorial and still unclear, linked to an abnormal intestinal immune response to the gut microbiota in genetically susceptible individuals [18,61].

Microbiota changes in IBD are characterized by a higher proportion of *Actinobacteria* and *Enterobacteria* (containing many of the gut pathogens) and decreased load of *Bacterioidetes* and *Firmicutes*, especially in regions of active inflammation [20,61]. The latter groups of bacteria are known to produce SCFA metabolites with potent anti-inflammatory activity [20,61].

Anti-inflammatory drugs (topical or systemic), such as aminosalicylates (e.g., 5-aminosalicylic acid (mesalamine) and its azo derivatives sulfasalazine, balsalazide, and olsalazine) and corticosteroids (e.g., prednisone, hydrocortisone) are the first approach in the treatment of IBD. In case of persistent disease activity, adverse events to aminosalicylates or severe refractory IBD, immunosuppressant drugs are used, such as thiopurines (azathioprine or 6-mercaptopurine), cyclosporine or tacrolimus, and ultimately biological agents like infliximab (a tumor necrosis factor (TNF)-α inhibitor), methotrexate, or natalizumab and vedolizumab (integrin inhibitors) [20,61,62].

Probiotics are promising agents for treating and preventing relapse of IBD due to their impact on host gut microbiota and mucosal immunoregulation via restoration of the balance between proinflammatory and anti-inflammatory cytokines [18,61]. Several RCTs showed benefits of a range of probiotics in IBD, particularly UC and pouchitis, while current evidence in CD is less promising [18,61].

3.3.1 Crohn's disease

In patients with CD, multiple studies comparing probiotics and placebo showed no significant difference in clinical outcomes [18,20,61], for instance, none of the *Lactobacillus* strains co-administered with steroids to adult CD patients showed any effect on endoscopic remission or on the Crohn's disease activity index (CDAI) scores [61]. The commensal bacterium *Faecalibacterium prausnitzii* found in lower numbers in patients with CD has been shown to

reduce proinflammatory cytokines in Caco-2 cell lines and to attenuate the severity of induced colitis in mice [3,61] with potential for the treatment of CD.

Small-sized studies showed a clinical benefit of *S. boulardii* in decreasing stool frequency and improving CDAI scores, accompanied by a decrease in the rate of clinical relapse as measured by the CDAI [61,63]. For CD patients in remission treated with the yeast, the lactulose/mannitol ratio used to measure intestinal permeability decreased in the probiotic group compared with placebo, suggesting a beneficial effect [61,63]. However, a larger trial failed to provide any clinical benefit of *S. boulardii* in relapse rates for CD patients in remission after salicylate or steroid therapies, according to the CDAI score [61,63].

Very recently, the probiotic mixture VSL#3 has been evaluated as an alternative to ineffective single-strain probiotic formulations in the prevention of CD recurrence after surgery [63,64]. Patients with CD were given either placebo or 1 sachet of VSL#3 (900 billion viable bacteria) within 30 days of ileocolonic resection and re-anastomosis. After 3 months, patients receiving VSL#3 had reduced mucosal levels of inflammatory cytokines compared with those receiving placebo, although there were no statistical differences in endoscopic recurrence between the two groups. However, VSL#3 was found to exert 12-month protective benefit in patients that started therapy immediately after surgery compared with those starting it after 3 months of placebo treatment, which warrants further investigation of VSL#3 as a potential probiotic agent for CD [63,64].

Altogether, there is not enough evidence for a beneficial role of probiotics in induction or maintenance of remission in CD [3,61,63].

3.3.2 Ulcerative colitis

Probiotics, either alone or in addition to standard IBD therapy, have shown promising results in induction and maintenance of remission of UC.

Bifidobacteria-fermented milk, containing *Bifidobacterium* strains (*B. breve* and *B. bifidum*) combined with *L. acidophilus*, showed significant improvement in cytokine profile, Clinical Activity Index (CAI) and histological scores when compared with placebo or no additional treatment [61]. On the other hand, a triple combination of *B. longum*, *L. acidophilus* and *E. faecalis* improved clinical symptoms and colonic mucosal inflammation in UC patients when combined with 5-aminosalicylic acid [61].

On the other hand, the non-pathogenic strain *E. coli* Nissle 1917 was found to be equivalent to mesalamine for UC remission and maintenance, both in adults and in children [20]. Similarly, no difference in preventing relapse of UC has been found between the probiotic (10^{11} CFU/d) and standard mesalamine therapy (2.4 g/d) when combined with prednisone and oral gentamicin [20].

Ambulatory adult patients and children with mild to moderate UC not responding to conventional therapy showed induction of remission after treatment with the multistrain probiotic VSL#3, without adverse events [20]. Regarding the effect of VSL#3 in the pediatric population, administration of the probiotic mixture to children with acute UC, along with concomitant steroid induction and mesalamine maintenance therapy, led to remission in 92.8% of treated patients compared with only 36.4% in the placebo group, with relapse rates of 21.4% and 73.3%, respectively, within 1 year of follow-up [20,61]. Overall, probiotics may be beneficial in mild-to-moderate UC as adjuvant therapy [3,20].

3.3.3 Pouchitis

Pouchitis is an iatrogenic condition that occurs in approximately 50% of patients following proctocolectomy with ileal pouch-anal anastomosis for chronic UC. Broad-spectrum antibiotics, such as ciprofloxacin and metronidazole, are the standard therapy for pouchitis [61,65].

According to a recent Cochrane review, no difference was observed in clinical improvement between LGG and placebo for the treatment of acute pouchitis, neither between *B. longum* and placebo for prevention of pouchitis [65]. On the other hand, a pooled analysis suggested that VSL#3 was more effective than placebo for maintenance of clinical remission in chronic pouchitis [3,65]. Moreover, 90% of patients on the VSL#3 probiotic group had no episodes of acute pouchitis during the 12-month follow-up compared to 60% in the placebo group, suggesting a preventive role for the probiotic [3,65].

Evidence from systematic reviews and meta-analysis suggests that probiotics (particularly VSL#3) can provide considerable benefit in the treatment of acute pouchitis and maintenance of clinical remission [61,65].

3.4 Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a GI disorder characterized by the association between recurrent abdominal pain or discomfort and a change in stool consistency or frequency [66] whose etiology remains unclear. The pathophysiology of IBS is multifactorial and has been linked to alterations of the gut microbiota, small-bowel bacterial overgrowth, and abnormal function along the brain-gut axis associated with GI hypersensitivity, motor dysfunction, and deregulated mucosal immune responses directly related to deterioration of intestinal barrier function, leading to disease symptoms like discomfort and pain, diarrhea, constipation, or alternating bowel movements [12,66].

There is no cure for IBS and treatment focuses on the relief of symptoms. Changes in diet and lifestyle are often sufficient to control mild IBS but moderate and severe IBS may require medication. Fiber supplements are recommended for constipation and anti-diarrheal drugs for the control of diarrhea, while anticholinergic and antispasmodic drugs can help relieve painful bowel spasms. Antidepressant drugs have also been used when IBS symptoms include abdominal pain or depression. Antibiotics are used in IBS cases associated with intestinal bacterial overgrowth [66]. Most of these drugs have been associated with adverse events, such as diarrhea, constipation and bloating.

Probiotics are likely to play a role in amelioration of IBS symptoms due to their impact on the gut microbiota [21]. Furthermore, several studies showed a luminal dysbiosis in IBS patients characterized by decreased lactobacilli and bifidobacteria, the genera frequently used in probiotic products, which can help replenish these species [66]. Probiotics can exert an additional beneficial effect on the intestinal mucosa through suppression of the growth and binding of pathogenic bacteria, modulation of the host immune response and enhancement of mucosal integrity, with improvement of bowel dysmotility [21]. Results from *in vitro* studies and from *ex vivo* cell cultures, as well as from several animal models (mice and rats) in which the epithelial barrier integrity had been disrupted before administration of probiotics, showed alleviation of IBS-like symptoms and restoration of the barrier function [21].

A systematic review of 19 RCTs with a total of 1,650 IBS patients showed that probiotics were significantly superior to placebo in the relief of IBS symptoms and improvement of quality of life (QoL) but failed to identify the most beneficial species and strain [67]. A more recent meta-analysis [68] confirmed the beneficial effects of probiotics, namely statistically significant improvements in terms of global symptom, abdominal pain, bloating and flatulence scores. *L. plantarum* 299v (DSM 9843) or probiotic combinations were the most effective [68–70].

The dosage in probiotic formulations is also relevant for the therapeutic effect. In a clinical trial conducted with 362 women with IBS treated with either placebo or probiotic capsules containing *B. infantis* 35624 at a dose of 10^6, 10^8 or 10^{10} CFU/capsule for 4 weeks, only the 10^8 CFU dosage was superior to placebo in the relief of IBS symptoms, particularly pain and discomfort [68,71]. The higher dosage (10^{10} CFU) was associated with low bioavailability due to formulation problems, namely resistance to dissolution [68,71]. On the other hand, this same dosage had proven effective when provided in a milk-based formulation to IBS patients, where the beneficial effects of *B. infantis* 35624 were associated with restoration of the balance between proinflammatory and anti-inflammatory cytokines [68,71].

Overall, probiotics are effective in amelioration of IBS symptomatology, showing both strain-specific and dose-dependent effects.

4. Safety of probiotics

Probiotics are generally recognized as safe (GRAS) by the Food and Drug Administration (FDA). Traditional LAB, which are normal colonizers of the human body and prevalent in fermented food, have a safety record of human consumption. Bifidobacteria share a similar safety profile [19]. However, the therapeutic use of probiotics is recommended only in specific situations and for certain probiotic strains at appropriate doses.

Probiotics are usually intended for the otherwise generally healthy population and several studies have documented the safety of short-term probiotic interventions in immunocompetent individuals [39,72]. Moreover, no increased incidence of lactobacilli-induced bacteremia has been observed after the introduction and widespread use of dairy products supplemented with probiotic *Lactobacillus* in Northern European countries, according to population-based studies [53].

However, probiotic use by preterm infants, immunocompromised and chronically ill individuals requires caution and the risk-benefit ratio must be carefully considered before recommendation [73]. There are also several underlying risk factors, such as indwelling catheters and GI disorders associated with compromised gut permeability and/or immunity that can enhance bacterial/fungal translocation and predispose to probiotic sepsis [40].

There is a risk of fungemia with *S. boulardii* in immunocompromised individuals and sporadic infections, including bacteremia, septicemia, pneumonia and deep abdominal abscesses, have been reported in neonates, severely debilitated and immunocompromised patients, such as those with HIV, severe neutropenia, and cancer, as a result of probiotic administration [74]. On the other hand, there are studies reporting the safety of probiotics in preterm neonates, and in immunocompromised children and adults with HIV [38]. Endocarditis has been reported in patients with damaged or artificial heart valves treated with lactobacilli [74]. There are also documented cases of adverse events that resulted from probiotic use by patients under enteral or parenteral nutrition with pancreatitis or undergoing transplant [72]. Therefore, it is advisable to avoid probiotic use in populations at risk for adverse events until further trials that produce robust data on probiotic safety have been conducted.

5. Conclusions

Current evidence supports the role of probiotics in a broad range of GI disorders, the best documented case being the prevention and treatment of acute infectious diarrhea. Future studies addressing safety, identity, stability and GI

survival of probiotics are needed to identify the optimum strain(s) or strain combinations for specific GI disorders, optimum doses and duration of therapy. Moreover, mechanisms of action should be clarified before translational to the clinical setting.

The clinical efficacy of a probiotic product depends on the specific strain, dosage, formulation, residence time in the gut, microbial viability on the shelf and in the intestine. Thus, it is difficult to make recommendations concerning specific probiotic products unless clinically tested in its final formulation and marketed dosage. Additionally, the quality, composition, and formulation among probiotic products vary widely, mainly due to lack of regulation of the probiotics market since mostly all probiotic products are marketed as foods or dietary supplements. Probiotics are mainly targeted to the generally healthy population and although empirical evidence suggests there is minimal potential for harm with probiotics, due caution is recommended in the administration of probiotics to immunocompromised individuals.

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