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Saccharomyces boulardii: a summary of the evidence for gastroenterology clinical practice in adults and children

R. BERNI CANANI[1], S. CUCCHIARA[2], R. CUOMO[3], F. PACE[4], F. PAPALE[5]

[1]Department of Pediatrics and European Laboratory for the Investigation of Food Induced Diseases, University of Naples "Federico II", Naples (Italy)
[2]Department of Pediatrics, "Sapienza" University, Rome (Italy)
[3]Department of Clinical and Experimental Medicine, University of Naples "Federico II", Naples (Italy)
[4]Department of Clinical Sciences "L. Sacco", University of Milan and Gastrointestinal Unit, "Bolognini" Hospital, Seriate (BG) (Italy)
[5]Field Medical Advisor, Zambon Italia, Bresso (MI) (Italy)

Abstract. – Probiotics are viable, non-pathogenic microorganisms (bacteria or yeast) which when administered in adequate amounts, confer a health benefit on the host. At this time, *Saccharomyces boulardii* is the only yeast commonly used in clinical practice. Literature on this probiotic is wide and even more data become available each year. Thus, it could be problematic for a physician summarize all the best information deriving from basic research and clinical studies. With the aim to help physicians in the use of *Saccharomyces boulardii*, this paper focuses on the available evidences for its efficacy and safety in different diseases in adult and pediatric patients in order to provide a practical guidance for gastroenterology clinical practice. Indications and dosage for several gastrointestinal diseases for a correct use of this probiotic are provided, and recent insights on its mechanisms of action and possible future clinical application are also discussed.

Key Words:

Probiotics, Acute gastroenteritis, Diarrhea, Irritable bowel disease, Inflammatory bowel diseases, *Clostridium difficile*, Antibiotic-associated diarrhea, Traveler's diarrhea, *H. pylori* infection.

Introduction

Probiotics are viable, non-pathogenic microorganisms (bacteria or yeast) which when administered in adequate amounts, confer a health benefit on the host[1]. More commonly used bacterial probiotics include *Lactobacillus* species, *Bifidobacterium* species, *Escherichia (E.) coli*, *Streptococcus* species, and the yeast *Saccharomyces boulardii* (*Sb*)[1-3]. With the increasing availability and wide spreading use of probiotics in gastroenterology clinical practice, it is important to assess which are the most effective preparations. A first problem for a physician could derive from the exact identification of the microorganism (strain and concentration) stated on the label of a particular product. At this time, *Sb* is the only yeast commonly used in clinical practice. The taxonomy of *Saccharomyces* strains has been debated, and in particular *Sb* strain has been questioned as to whether it should be reclassified as a strain of *S. cerevisiae* or remain a separate species[4]. In recent years, due to the availability of metabolomic tools (microsatellite polymorphism analysis and retrotransposon hybridization analyses), it has been shown that *Sb* has a unique clustering different from other strains of *S. cerevisiae*[5]. In addition, *Sb* can be distinguished from other strains of *S. cerevisiae* by advanced typing methods, by differences in metabolism and physiology and by the ability to have anti-microbial effects[6,7]. There are many different *Sb* products commercially available, which are sold either as lyophilized or heat-dried powders in capsules, or in liquid beverages[7]. The quality of these products is variable, and although most products state they contain at least 1×10^9 cfu/mg, independent assays have determined that about 50% of the products contained less[7]. In one study comparing six *Sb* products, all had

Corresponding Author: Roberto Berni Canani, MD, PhD; e-mail: berni@unina.it

identical PCR typing profiles, but only 50% of them had the same concentration identified on their label[8]. Differences in clinical efficacy may occur due to lower than stated dose but also to inaccurate strain composition, which may be due either to probiotic manufacturing technique (shelf-life), to the specific kind of preparation or ultimately to the use of probiotic mixture rather than single strain preparation. All the randomized controlled trials using *Sb* have utilized a single strain preparation[7]. Although mixtures of probiotics, which may contain *Sb*, are available on the market, there is no evidence from randomized controlled trials that these mixtures are superior to the single strain preparations. Moreover, potential limitation of probiotic mixtures is antagonism between the different probiotic strains and/or conflicting mechanisms of actions that may tend to attenuate the therapeutic responses of the probiotic strains[9]. What is now clear is that the efficacy of probiotic preparations for treatment of gastrointestinal diseases is related to individual microbial strains and doses. We believe that probiotic preparations should be regarded as drugs and physicians should select those preparations for which evidence of efficacy, or of greater efficacy in a given clinical condition, is supported by solid data. Literature on *Sb* is wide and even more data become available each year; thus, a second problem for a physician could derive from the difficulty to summarize all the best informations deriving from basic research as well as from clinical studies. With the aim to help physicians in the use of *Sb*, this paper focuses on the available evidences for its efficacy and safety in different diseases in adult and pediatric patients in order to provide a practical guidance for gastroenterology clinical practice.

To do this PubMed, Medline, and Google Scholar were searched for articles unrestricted by language from 1983 to March 2011. Search term included *Sb,* probiotics, gastroenterology, gastrointestinal tract, gut, intestine, infections, children, pediatric patients, adult patients.

Mechanisms of Action of *S. boulardii*

A very active research in this field has provided interesting data on several mechanisms of action of *Sb*. They may be classified into three main areas: anti-microbial action, trophic action, and immunoregulation.

Anti-Microbial Action

Within the intestinal luminal *Sb* exerts several anti-microbial activities that could be divided in two groups:

Direct anti-toxin effects. The anti-toxin action elicited by *Sb* is mainly due to small peptides produced by the yeast. A 54kDa serine protease is able to inhibit enterotoxin and cytotoxic activities of *C. difficile* by degradation of toxin A and B and receptors sites of toxin A on the enterocyte cell surface. Others *Saccharomyces* strains fail to show these activities[10,11]. A 120 kDa protein that has a non-proteolytic activity, competes specifically with the hyper-secretion caused by the toxins of *Vibrio (V.) cholera* decreasing cyclic adenosine monophosphate in the enterocytes[12,13]. Finally, *Sb* produces a phosphatase able to dephosphorylate endotoxins (such as lipopolysaccharide of *E. coli* 055B5) and inactivates its cytotoxic effects[14]. This mechanism may account for the protection afforded in cases of sepsis.

Inhibition of growth and invasion of pathogens. *In vitro*, *Sb* directly inhibits the growth of several pathogens (*Candida albicans, E. coli, Shigella, Pseudomonas aeruginosa, Staphylococcus aureus, Entamoeba hystolitica),* and cell invasion by *Salmonella typhimurium* and *Yersinia enterocolitica*[15-17]. The yeast may also act by enhancing the integrity of the tight junction between enterocytes, thus preserving intestinal integrity and function[18,19]. *S. boulardii* is also able to reduce the translocation of pathogens in rat and pig animal models[20-22], and it can also interfere with pathogenic attachment to intestinal receptor sites[19,23,24]. In enteropathogenic *E. coli* (EPEC) infection, *Sb* acts as a decoy by causing bacterial cells to directly bind to its surface rather than to enterocytes[25]. *In vitro*, *Sb* inhibits the adhesion of *C.albicans* to epithelial cell lines, this effect is also observed with the extracts of *Sb*[26]).A study suggests the capric acid as factor secreted by *Sb* and responsible for inhibition of *C. albicans* filamentation and partially also adhesion and biofilm formation[27]. Contrasting data derives from a recent study in a murine animal model suggesting that the oral administration of *Sb* does not prevent gastrointestinal colonization by *C. albicans*[28]. Finally, data on the effects of *Sb* against common viruses responsible for diarrhea (such as *Rotavirus, Aden-*

ovirus, Norovirus) are still very limited, and further research in this field is advocated also considering the epidemiologic importance of these pathogens in clinical practice[29].

Trophic Action

When *Sb* is given to antibiotic-shocked animal or patients with diarrhea, normal microbiota is re-established more rapidly. On the contrary, *Sb* has no effect on microbiota composition in healthy humans[30-32]. This effect is tightly linked to a stimulation of short chain fatty acids (SCFA) production, especially butyrate[30,33,34]. The production of SCFAs is significantly decreased in patients receiving antibiotics. The effect on butyrate production is particularly relevant considering the important role of this compound for the regulation of many intestinal functions including, the stimulation of enterocytes growth and differentiation, fluid absorption, immune stimulation, anti-inflammatory effects, enteric neurons growth and differentiation[34]. These effects may be especially relevant in the management of antibiotic-associated diarrhea (AAD). *S. boulardii* exerts trophic effects and enhances enzymes expression on microvilli of the host, it improves disaccharidases activity, enhance the absorption of D-glucose coupled to Na^+ by the symport glucose/Na^+ and expression of the sodium-glucose cotransporter-1 (SLGT-1)[35] The yeast stimulates the activity of sucrase at levels high enough to be effective in the treatment of congenital sucrase-isomaltase deficiency[36,37]. *S. boulardii* stimulates mucosal peptidase activity and endoluminal peptide hydrolysis in suckling rat small intestine, and it was showed that this yeast is also able to produce and secrete in the intestinal lumen a leucine aminopeptidase, belonging to the Zn^{2+}-metalloprotease family, with proteolytic activity on endoluminal N-terminal of oligopeptides. This effect could be potentially important in preventing reactions to food antigens when mucosal permeability is increased[38]. *S. boulardii* stimulates the production of glycoproteins in the brush border of microvilli such as hydrolases, transporters, secretory IgA, and the receptor for polymeric immunoglobulins[39]. The production of intestinal polyamines induced and stimulated by *Sb* is one of its most relevant and specific mechanisms of action. The polyamines spermidine, spermine, and putrescine enhance the expression of brush border enzymes (such as hydrolases, proteases, and transport molecules)[35,40]. In addition, *Sb* activates expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) that protects from gut inflammation and inflammatory bowel diseases of the host[41].

Immunoregulation

The toxins elaborated by *Clostridium difficile* and pathogenic bacteria, such as EPEC and enterohaemorragic *E. coli* (EHEC), activate the MAP kinases ERK 1/2 and p38 as well as the NF-kB (p65/p50) system leading to transcription of pro-inflammatory genes such as interleukin 8 (IL-8) that promote inflammation. *S. boulardii* inhibits MAP kinase and NF-κB signal transduction pathways and decreases the secretion of IL-8 and reducing inflammatory diarrhea. *S. boulardii* also secretes a small (<1 kDa) heat-stable and water-soluble anti-inflammatory factor termed *"S. boulardii anti-inflammatory factor"* that inhibits the NF-κB-dependent signaling pathway in the presence of *C. difficile* toxin[42]. The yeast also decreases enterocyte apoptosis, probably as a result of the decrease of the synthesis of TNF-α[43]. It has been recently postulated that *Sb* inhibits dendritic cell-induced activation of naïve T cells[44]. It also modifies the migration of lymphocytes in a model of inflammatory bowel disease (IBD)[45]. Recent research demonstrated that the supernatant of *Sb* cultures modifies the capacity of lymphocytes to adhere to endothelial cells, leading to improved cell rolling and adhesion[45]. There is a very clear and marked stimulation of production of IgA and specific IgG anti-toxins A and B by *C. difficile*, as demonstrated by the increased content in the intestinal lumen and in crypt cells. This may be explained by a trophic effect exerted on the mucosa or by direct immunostimulation[39]. Another mechanism indirectly involved in the immune-regulation effect exerted by *Sb* is the modulation of intestinal permeability. Increased intestinal permeability is frequently observed in different situations such as following shock, burn injury, obstructive jaundice, intestinal resection, hepatic transplant, or intestinal obstruction (IO)[46-50]. A recent study has demonstrated that in a murine IO model the oral pretreatment with viable or heat killed cells of *Sb*, preserves intestinal integrity and modulates inflammation, preventing bacterial translocation and intestinal lesions[51]. Finally, it has been demonstrated that *Sb* modulates the nitrogen oxide pathway through the inhibition of the iNOS, contributing to a general down-regulation of intestinal inflammation and an anti-secretory stimuli on transepithelial ion transport[52].

Evidences of Clinical Efficacy in Adults

There are several randomized controlled trials testing *Sb* in adult patients and mostly found a significant protective effect elicited by this probiotic. Areas of interest include acute diseases (AAD, acute diarrhea, traveler's diarrhea, *C. difficile* infection, *Helicobacter (H.) pylori* disease, enteral nutrition-related diarrhea) and chronic conditions (Crohn's disease, irritable bowel syndrome, giardiasis).

Antibiotic-Associated, Acute and Traveler's Diarrhea

The efficacy of *Sb* in the prevention of AAD has been tested in ten randomized controlled trials (RCT)[7,53-60]. Eight of these (80%) showed a significant efficacy in the prevention of the disease. A total number of 968 patients have been treated with *Sb* (doses ranging between 4×10^9 to 2×10^{10} cfu/day). A meta-analyses of these ten studies showed that *Sb* was significantly protective for ADD with a risk of 0.47 (95% confidence interval 0.35-0.63) and the number needed-to-treat (NTT) in order to prevent one case of ADD was 10.2[7]. Among these studies, three trials have been carried out on patients with *H. pylori* infection receiving triple therapy, which is associated with a high rate of ADD development[53,54,58]. *S. boulardii* is able to decrease the prevalence of ADD due to triple therapy so as to eradicate *H. pylori* from 8.7% to 25% with respects to placebo control.

Only two randomized controlled trials using *Sb* showed that this probiotic may be effective in treating acute diarrhea. In the first trial *Sb* was able to improve the severity of diarrhea after 72 hours compared to placebo. The second study is a RCT showing the efficacy of *Sb* in the treatment of acute diarrhea caused by *Entamoeba histolytica*[61]. In this study, 57 patients with acute dysentery received metronidazole and iodiquinol for 10 days with and without *Sb.* All patients treated with the yeast were cured when compared to 19% of control patients. In a meta-analysis carried out in order to evaluate 12 RCTs of various probiotics for the prevention of traveler's diarrhea, two RCTs focused on *Sb* administered 5 days before the trip and continued through the duration of the trip showed the efficacy of this probiotic to reduce of traveler's diarrhea in a dose-dependent manner with the best effective daily dose of 2×10^{10} cfu[62]. No well-designed studies are available on the treatment of traveler's diarrhea.

C. difficile Infection

Four trials analyzed the use of *Sb* in *C. difficile* infection (CDI). Two double-blinded RCTs investigated the use of *Sb* and antibiotics for patients with recurrent CDI. In the first, three antibiotic regimens were used over a 10-day period (either a high or low-dose of vancomycin or metronidazole) combined with *Sb* or placebo. After a 2-month follow-up, a significant decrease of CDI recurrences was observed only in patients treated with a high-dose of vancomycin and *Sb* compared with antibiotic and placebo-treated patients[63]. In the second double-blinded RCT, the Authors verified a lower recurrence rate of CDI disease in 57 patients on varied doses of vancomycin or metronidazole and *Sb* (26.3%) compared with 67 patients treated with antibiotic and placebo (44.8%)[64]. The other two studies examined primary prevention of CDI in patient populations who were recently prescribed antibiotics but showed no signs or symptoms of CDI[55,65]. The presence of the *C. difficile* (verified by culture, or by the presence of toxin A and B) and clinical outcomes of diarrhea were recorded. However, these studies did not have the power to detect statistically significant differences. Because only a small number of studies address the primary prevention of CDI, more research is required before any changes in practice can be recommended with regard to using *Sb* in this setting[66].

Helicobacter pylori Eradication

Studies indicate that *Sb* may not be effective enough to eradicate *H. pylori* but it decreases the side-effects of the standard triple therapy. Indeed, two trials have been performed on adult patients. The first explored the efficacy of three probiotic treatments (*Sb*, *Lactobacillus rhamnosus GG* or a mixture of *L. acidophilus* and *Bifidobacterium lactis*) or placebo associated with the triple therapy for the first week in asymptomatic *H. pylori* carriers. At the end of the second week, *H. pylori* eradication rates were similar for all groups, but those regarding antibiotic-associated diarrhea were lower (5%) in probiotic groups compared to placebo (30%)[58]. The other study also assessed *Sb* for both the eradication of *H. pylori* and the reduction of side-effects relating to standard antibiotic treatment (53). This study involved 124 adults with *H. pylori* dyspepsia who were receiving the triple therapy and this was randomized using either *Sb* or placebo. After six weeks there was no significant difference in *H. pylori* eradi-

cation (71% in *Sb* vs 60% in placebo), whereas significantly fewer patients randomized to *Sb* reported epigastric distress (14.5%, $p < 0.05$) compared with placebo (43.5%), as well as lower global dyspepsia symptom scores.

Enteral Nutrition-Related Diarrhea

Diarrhea is a common complication associated with enteral feeding and this may result in a loss of nutrients in an already seriously ill patient. Three RCTs have assessed the ability of *Sb* to reduce diarrhea in patients receiving enteral nutrition. *S. boulardii* administered between 11 and 21 days was able to reduce diarrhea (8.7%) when compared to the placebo (16.9%)[67]. In the other two studies, patients under enteral nutrition treated with *Sb* for a time varying between 8 and 28 days had a significant fewer diarrheal episodes[68,69].

Crohn's Disease

Two randomized controlled clinical trials were carried out testing *Sb* in patients suffering from Crohn's disease. In one RCT, 20 randomized patients, continuing their maintenance therapy, were treated with either *Sb* or a placebo over a 7-week period[70]. At the end of the study, patients treated with *Sb* were significantly improved when compared with those treated with placebo (3.3±1.2 *vs* 4.6±1.9 stool movements/day). *S. boulardii* added to mesalamine therapy was also able to decrease the relapse rate in patients with Crohn's disease (n=32) observed over a 6-month period (*Sb*+mesalamine 6% *vs* mesalamine alone 38%)[71].

Irritable Bowel Syndrome (IBS)

A recent study was aimed to evaluate the effects on the quality of life (QOL) and symptoms in patients with diarrhea-predominant irritable bowel syndrome IBS or mixed-type IBS. Sixty-seven patients with IBS were randomized either to receive *Sb* or a placebo for 4 weeks. The overall improvement in IBS-QOL was higher in the *Sb* group than in the placebo-treated group (15.4% *vs* 7.0%; $p<0.05$). Composite scores for IBS symptoms were significantly reduced in both groups to a similar extent. Bowel frequency and stool consistency did not change in either group[72].

Giardiasis

S. boulardii has been also tested in giardiasis. This condition is characterized by long-lasting diarrhea with symptoms ranging from mild to severe diarrhea, weight loss, abdominal pain and weakness. In one study, 65 randomized adults were treated to receive either *Sb* or placebo for 10 days. Both groups also received metronidazole for the same period of time. Two weeks later, both groups reported a resolution of their diarrhea, but none of those on *Sb* had detectable *Giardia* cysts, while significantly more (17%) on placebo still carried *Giardia* cysts[73].

Other Gastrointestinal Diseases

Potential uses of *Sb* were studied as regards the treatment or the prevention of other conditions such as HIV-1-related diarrhea. Indeed, 35 patients with HIV-1-related diarrhea were randomized to receive either high daily doses of *Sb* (6×10^{10} cfu) or a placebo for one week. More of those patients given *Sb* (61%) had their diarrhea resolved when compared with placebo (12%)[74].

Indications regarding treatment with Sb were also hypothesized for *Blastocystis hominis* infections. Recent data suggest that these infections cause frequent symptoms and *Sb* is able to cure the infections in a comparable manner with respects to metronidazole[75].

Evidences of Clinical Efficacy in Children

The pediatric clinical conditions in which *Sb* has been investigated are mainly acute diarrhea, persistent diarrhea, AAD, *H. pylori* infection. Although its administration seems to have produced healthy benefits in most of patients treated, only few studies have been conducted in a well designed manner or as controlled trials.

Acute Diarrhea

As for other probiotic products, the effect of *Sb* on diarrhea is variable. It possibly depends on disease severity, different clinical settings and outcomes. Six RCTs investigated the potential efficacy of *Sb* in children with acute diarrhea, in 5 significant reduction of total diarrhea duration was demonstrated, in one no difference between *Sb* and the control group was observed. However, based on the pooled results of these six RCTs involving 756 children, *Sb* compared to placebo or no intervention, reduced the total duration of diarrhea by 22 hours (CI -26 to -18 hours). The data available in literature suggest that in otherwise healthy infants and children with acute infectious diarrhea (of viral or bacterial origin), the use of *Sb* compared to controls is associated with moderate therapeutic benefit that is reproducible re-

gardless of the outcome measure studied (i.e. duration of diarrhea, chance of cure or risk of diarrhea at certain point intervals, number of stools and length of hospital stay)[76]. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition and the European Society of Paediatric Infectious Diseases Expert Working Group stated that selected probiotics may be an effective adjunct to the management of diarrhea. However, because there is no evidence of efficacy for many preparations, only probiotic strains with proven clinical efficacy and in appropriate dosage are recommended as an adjunct for the management of children with acute gastroenteritis to rehydration therapy. *Lactobacillus GG* and *Sb* constituted examples of probiotics that showed benefit[77]. In a recent double-blind RCT children with acute *Rotavirus* diarrhea received one of two treatments (oral rehydration solution plus *Sb* or plus a compound containing *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum* and *Sb)* or placebo. Results showed that both products decreased the duration of diarrhea compared to oral rehydration solution alone, but only for the single species product (containing *Sb*) the decrease was significant, children in this group showed a decrease in fever duration[78]. Another recent trial evaluated the clinical efficacy and cost/effectiveness of *Sb* compared with yogurt fluid (YF) in acute non-bloody diarrhea in children. This randomized, prospective open-label clinical trial included 55 children (36 boys, mean age 21.2±28.2 months). Group A (n=28) received lyophilized *Sb* and group B (n=27) received YF. The duration of diarrhea was shorter with *Sb* but the hospital stay was reduced with YF, although these differences were not significant. However, on day 3 the rate of children with diarrhea was lower in the *Sb* group (48.5% versus 25.5%; $p < 0.05$)[79].

Antibiotic Associated Diarrhea (AAD)

Three RCTs investigated the potential efficacy of *Sb* in the prevention of AAD in children, in two a significant reduction of total prevalence of diarrhea was demonstrated[80]. In one no difference between *Sb* and the control group was observed. But it should be stated that in this study diosmectite was used in the control group. However, in the two studies showing an efficacy of *Sb* in the prevention of AAD a RR 0.3, 95% CI 0.2 to 0.5 and 0.3 95% CI 0.2 to 0.7, was observed respectively. Very limited data are available in children with AAD or with CDI treated with *Sb*[81]. A recent case report suggest the potential utility of *Sb* for recurrent CDI in a child with surgically treated Hirschsprung's disease. The patient had several intermittent episodes of CDI, and significant benefits were derived from *Sb* therapy, with reduction in frequency and severity of episodes. The child was more medically stable during the subsequent episodes, and there was less consequent disruption to the child's developmental and psychosocial well-being[82].

Persistent Diarrhea

Persistent diarrhea is defined by WHO as an episode which starts acutely but which persists for 14 days or longer. The main dangers of persistent diarrhea are malnutrition and severe extra-intestinal infections. The morbidity and mortality of persistent diarrhea and the magnitude of the problem, particularly in the developing world, justify any attempt to improve treatments[83]. To determine whether treatment with *Sb* improves outcomes in children with persistent diarrhea a RCT was conducted in 89 children (age range 6-24 months) allocated to receive pasteurized cow's milk supplemented with two viable lyophilized strains *Lactobacillus casei* and *L. acidophilus* strains 10^{10}–10^{12}cfu/g (n=30), or lyophilized *Sb* 10^{10}-10^{12}cfu/g (n=30), or pasteurized cow's milk as placebo (n=29), twice a day for 5 days. Enteric pathogens (R*otavirus, E. coli, Salmonella, Shigella*) were isolated from the stools in 40% of the patients, 27% had *Rotavirus*. *Lactobacillus* strains and *Sb* significantly reduced the number of stools per day ($p<0.001$) and diarrhea duration ($p<0.005$)[84]. Another trial was carried out in 40 children (aged 6 to 36 months) with diarrhea of 3 or 4weeks duration were randomized to receive *Sb* at a dose of $1x10^{10}$ cfu/day (n=20) or placebo (n=20) for 1 month. All patients received either tinidazole for *Giardia lamblia* (87.5%) or trimetoprim plus sulfamethoxazole for *Shigella* just prior to the intervention. At the end of the study, the percentage of children with <3 bowel movements per day was significantly higher in the *Sb* group compared to the placebo group (65% *vs* 15%, $p=0.002$)[85]. Taken together, these results suggest that *Sb* could be useful in the management of persistent diarrhea in children.

Helicobacter pylori Infection

S. boulardii plus inulin could hold promise as an adjunctive agent to the triple therapy. This is based on the results of one open RCT evaluating whether consumption of either the combination

of *Sb* plus inulin or of *L.acidophilus* LB would affect *H. pylori* eradication in the pediatric population. Two hundred and fifty-four asymptomatic children, who were positive for *H. pylori* infection by the ^{13}C-urea breath test, were allocated to one of three groups: (1) 8-day course of eradication triple therapy with lanzoprazole, amoxicillin, and clarithromycin; (2) *L.acidophilus* LB; and (3) a symbiotic (*Sb* plus inulin). *H. pylori* was eradicated in 66% (30/45), 12% (6/51), and 6.5% (3/46) of the children from the antibiotic group, symbiotic group, and probiotic groups, respectively ($p<0.001$)[86]. Although the eradication rate of *H. pylori* with *Sb* is low, further studies elucidating the mechanisms by which *Sb* with or without additional inulin may inhibit *H. pylori* would be of interest.

Blastocystis Hominis Infection

The pathogenic potential of *B. hominis* remains controversial and up to now there is a consensus that therapy should be limited to patients with persistent unexplained symptoms after an accurate evaluation and a complete negative screening for alternative etiologies. Metronidazole is the most recommended agent in the treatment for *B. hominis* infection[87]. Also if some data suggest no beneficial effects for blastocystosis (88). A recent study has demonstrated the potential beneficial effects in *B. hominis* infection (symptoms, presence of parasites) in children treated with metronidazole or *Sb*. Three groups were analyzed: group A, treated with *Sb* (250 mg twice a day) for 10 days; group B, treated with metronidazole (30 mg/kg twice daily) for 10 days; and for group C no treatment was provided. On day 15, clinical cure was observed in 77.7% in group A (n=18); in 66.6% in group B (n=15); and 40% in group C (n=15) ($p<0.031$, between groups A and C). Disappearance of the cysts from the stools on day 15 was 80.0% in group B, 72.2% in group A, and 26.6% in group C ($p=0.011$, between group B and group C; $p=0.013$, between group A and group C). At the end of the first month after enrolment, clinical cure rate was 94.4% in group A and 73.3% in group B ($p=0.11$). Stool presence resulted negative for *B. hominis* in a similar way in both groups (94.4% vs. 93.3%, $p=0.43$)[89].

Amebiasis

In a recent study the addition of *Sb* (0.5×10^{10} cfu/mg) to metronidazole for a 7-day treatment of acute bloody diarrhea due to intestinal caused by *Entameba histolytica* resulted in a significant decrease of diarrhea duration and enhance of the gastrointestinal clearance of the amebic cysts, compared to treatment with metronidazole alone[90].

Pharmacokinetics

S. boulardii is generally administered in lyophilized powder corresponding to approximately 3×10^{10}cfu/gr. Unless exogenously administered it is not found as part of the intestinal microflora of humans and under normal conditions it doesn't permanently colonize the gastrointestinal tract. Daily administration as lyophilized powder guarantees vital persistence of yeast along the whole gastrointestinal tract. In fact, this yeast, because of its peculiarity it's not absorbed, it has no systemic impact, is resistant to gastric acids and to bile secretions and, within 48-120 hours since the end of treatment, at usual doses, is not anymore detectable in the stools. Pharmacokinetics studies indicate that *Sb* is able to reach fast and high concentrations in the colon without colonizing it[91]. In human adult volunteers, following a single oral 1g dose maximal stool concentration was achieved between 36 and 60 hours post-dose and after 2-5 days the levels were below the limit of detection[92].

Stability

Probiotic product manufacturing may affect its shelf-life. Probiotics may be available as lyophilized or heat-dried preparations or contained in diary or drink food products. Heat-dried capsule products may be identified by their labels, which usually state that the products should be refrigerated after opening and they lose their potency rapidly. Lyophilized preparations of *Sb* are stable over one year at room temperature, as long as it is protected from moisture[93], and have the advantage of portability and convenience and maintain high viability counts over prolonged periods. Heat-dried preparations are not stable at room temperature and must be refrigerated[7].

Safety

Safety and adverse event data collected have documented a remarkable safety profile for *Sb* in otherwise healthy patients (adults and children) with AAD, infections of the gastrointestinal tract,

IBD, IBS receiving this yeast by oral route. Also in *HIV-1*-infected patients the therapy with Sb resulted to be safe[74]. Infrequent cases of fungemia have been reported in case reports or case series. Most of them presented severe co-morbidities and have central venous catheters. In some of these patients yeast was acquired from contaminated environmental fomites[94]. *S. boulardii* in the formulations actually on the market in Europe are gluten free, and may be administered also during pregnancy and suckling. Finally, also if the risks of administering *Sb* seem to be minimal compared with placebo, adverse reactions are not excluded. A case report described the occurrence of allergic reaction caused by *Sb*[95], and patients receiving treatment with *Sb* for the prevention of CDI experienced increased risk for thirst and constipation[7].

Contraindications/Precautions

Use in patients who have central venous catheters is generally contraindicated. Unlike many bacterial probiotics, there are few drug or antibiotic interactions with *Sb*. However, if patients are on anti-fungal medications, a staggered dose regime (at least 4 h apart) is suggested[96]. A critical cost/benefit ratio should be considered before the use of probiotics in severely ill patients, for a possible increased risk of systemic infections.

Future Research Perspectives and Conclusions

S. boulardii has been used as probiotic since 1950, and it has been investigated in *in vitro* studies in different experimental models and in several clinical trials worldwide in adult as well in paediatric patients. Not many other examples of a similar amount of data on a single probiotic is currently available in the scientific literature. The use of *Sb* as preventive and therapeutic strategy is supported by convincing data on its mechanism of action, pharmacokinetics, and efficacy in several conditions. A summary of indications and recommended doses for the clinical use of *Sb* in gastroenterology clinical practice is provided in Table I. The strength of evidence is high for the prevention of AAD and for TD in adult pa-

Table I. Summary of the main clinical indications and recommended doses for the clinical use of *S. boulardii* in gastroenterology clinical practice.

Disease	Daily Doses – cfu/day (mg/day)	
	Adult patients	Pediatric patients
Prevention		
Antibiotic-associated diarrhea	1-2 × 10^{10} (500-1000) during the entire duration of antibiotic therapy plus 3 d to 2 wks after	1 × 10^{10} (500) during the entire duration of antibiotic therapy plus 7-9 d after
Traveler's diarrhea	0.5-2 × 10^{10} (250-1000) for 5 d prior the trip and continued through the duration of the trip	
Enteral nutrition-related diarrhea	4 × 10^{10} (2000) for 8-28 d	
H. pylori eradication therapy side-effects	2 × 10^{10} (1000) for 2 wk	
Relapses in Crohn's disease	1.5-2 × 10^{10} (750-1000) up to 6 mo	
Treatment		
C. difficile infection	2 × 10^{10} (1000) for 4 wk	
Acute diarrhea	1.5-2 × 10^{10} (750-1000) for 8-10 d	< 1y of age 0.5 × 10^{10} (250) for 5-6 d > 1y of age 1 × 10^{10} (500) for 5-6 d
Irritable bowel syndrome	1 × 10^{10} (500) for 4 wk	
Giardiasis	1 ×1 0^{10} (500) for 4 wk	
HIV-related diarrhea	6 × 10^{10} (3000) for 7 d	
Persistent diarrhea		1 × 10^{10} (500) for 1 to 4 wk

Note: 1 × 10^{10} cfu of *Sb* correspond to 500 mg of dried power of the yeast in the commercially available products. *Legend:* d = day; wk = week; mo = month; y = year.

tients, and for the treatment of acute and persistent diarrhea and prevention of AAD in children. More studies are encouraged for other conditions referred in the Table II and for a better definition of mechanisms of action of this probiotic in selected diseases of the gastrointestinal tract. The quality of products available on the market is generally good but more research could be necessary to obtain new formulations even more stable, efficacious and practical for a wide clinical use even in pediatric patients, such as drops. In this light, are of particular interest recent data on new formulation of *Sb*, encapsulated in food-grade whey protein isolate (WP) and alginate (ALG) micro-particles, in order to protect and vehicle the yeast in gastrointestinal environment[97]. We know that a large proportion of the yeast is inactivated after oral administration[98], the gastrointestinal survival of the yeast in these micro-particles resulted significantly increased (40% *vs* 10%) and was further improved to 60% by coating. Coated WP/ALG micro-particles appear to have potential as oral delivery systems for *Sb* in pharmaceutical or food processing applications, and because this encapsulation system requires only food-grade components is, therefore, compatible with pharmaceutical requirements in terms of safety and biocompatibility. Recently, beneficial effect are demonstrated when the combined administration of *Sb* with zinc are used in the treatment of *Rotavirus* diarrhea in hospitalized children[99]. Zinc is a potent agent against infectious diarrheas[100], and the results of this study suggests the potential utility of a new formulation of *Sb* containing zinc that could result in a even more effective treatment for these very common disorders. This research together with new investigation on the use of *Sb* in the early infancy, including the neonatal period, could open the way to new possible clinical indications for prevention and treatment of pediatric diseases. For example, it has been recently demonstrated that children affected by cow's milk allergy, the most common food allergy in the pediatric age worldwide, have very few amounts of *Sb* in their intestinal microflora compared to healthy controls[101]. Thus, also considering the several immunoregolatory effects exerted by *Sb* at intestinal level, it could be interesting to investigate the efficacy of this probiotic in the prevention or treatment of food allergies. Finally, isolated yeast components could also be administered as therapeutics. Although this approach extends beyond the official definition of probiotics (defined as living microbes), it does not differ much in concept from vaccination, the administration of microbial components to stimulate selected immune functions. The use of nonviable yeast or yeast components would also avoid the risk of sepsis or other potential complication of probiotics, particularly in critically ill patients with compromised intestinal barrier function. As stated above, *Sb* produces several peptides and other compounds able to exert immune and non-immune effects of potential clinical importance also when administered alone, and this could be open the way to an innovative *"pharmacobiotical approach"* in human medicine deriving from this yeast. Strictly correlated to these possible approaches are the interesting data deriving from investigation on the effect of fermentation with *Sb*. A recent study suggest the utility of fermentation with *Sb* on rice bran. This study showed that different rice varieties fermented with *Sb* are able to reduce the growth of human B lymphomas compared to each variety's non-ferment-

Table II. The research agenda for future studies for a better definition of the *S. boulardii* use in gastroenterology clinical practice.

Clinical indications	Mechanisms of action
• Acute diarrhea in adults	• *Rotavirus* and other virus-induced diarrheal disorders
• Traveler's diarrhea	• Structure and effects of peptides and other compounds produced by *Sb*
• Inflammatory bowel disease	• Effects of mixtures of probiotics containing *Sb*
• Irritable bowel syndrome	• Effects of products containing *Sb* plus zinc
• *H. pylori* infection	• Effects of the addition of *Sb* to foods regarding the bioavailability of antigen epitopes and micronutrients
• Persistent diarrhea	
• *C. albicans* infection	
• Food allergy	

ed control and revealed that fermentation differentially altered bioactive compounds. This research could open the way to new studies exploring the effects of the addition of *Sb* to foods regarding the bioavailability of new compounds with biological effects. And this could be also of importance regarding the potential modulation of antigenic epitopes and micronutrients bioavailability deriving from the fermentation with *Sb* of certain foods. This approach has been suggested for food allergies and for celiac disease using different probiotics, but data on *Sb* are still limited[102]. Finally, probiotics are currently used in many baby foods with the aim to improve the wellness of the consumers protecting them from several diseases, in particular from infectious or immune-mediated disorders, but also in this case the experience with *Sb* is still limited.

In conclusion, studying well established probiotics (such as *S. boulardii*) basic scientists, clinical researchers and industrial leaders have the opportunity to work together against different diseases of gastrointestinal tract with great social and economic impact. Some beneficial effects of *Sb* need to be better defined and the molecular mechanisms that underlie these effects need to be investigated in detail. The compounds mediating these effects should be purified and identified, and may eventually be used for pharmaceutical purposes. Synergistic effects should also be investigated, together with the positive contribution of specific prebiotics. This yeast has an additional quality: it generally can be grown in cheap products and foods and, therefore, can contribute to gastrointestinal disease cure and prevention in both developed and developing countries.

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