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Benefits of multistrain bacteria formulations for health

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ABSTRACT

Gut microbiota dysfunction is associated with multiple gastrointestinal and extra-gastrointestinal diseases, thus the possibility of its modulation with prebiotics, probiotics and symbiotics has gained interest in the last years. Many probiotics are available on the market and products characterized by combination of multiple strains have been proposed as particularly effective. However, evidences supporting their efficacy are often inadequate and not homogenous. We reviewed available data on clinical efficacy of multistrain probiotics and symbiotics in gastrointestinal diseases in adults, focusing on data from randomised, double-blind, placebo-controlled clinical trials. Twenty-nine multistrain products satisfied inclusion criteria. The principal areas of application are irritable bowel syndrome, *Helicobacter pylori* eradication and antibiotic-associated diarrhoea. The most represented probiotic species in the different probiotic formulations was *L. acidophilus*. The combination of *L. paracasei, L. plantarum, L. acidophilus, L. delbrueckii* subsp. *bulgaricus, B. longum, B. infantis*, *B. breve* and *S. thermophilus* (VSL#3) showed the highest number of RCTs.

1. Introduction

Gut microbiota alterations and gut barrier dysfunction have been associated with multiple gastrointestinal and extra-gastrointestinal diseases. Thus, gut microbiota as possible disease modifier has gained interest in many field of medicine. Gut microbiota modulation is possible through many ways: diet modification, antibiotics, prebiotics, probiotics and, more recently, faecal microbiota transplantation (FMT). However, the use of antibiotics is limited by the possibility of inducing antibiotic resistance, whereas FMT is not largely available and it is still under investigation for indications other than *C. difficile* infection (Ianiro, Bibbo, Gasbarrini, & Cammarota, 2014). Differently, the use of probiotics is very diffused in routine clinical practice, due to the good safety profile and the large availability of multiple products on the market. However, there is still confusion in probiotic field regarding the appropriate use and definition of probiotics. In fact, probiotics are generally defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" according to the last FAO/WHO definition (2001) ("Food and Agricultural Organization of the United Nations and World Health Organization.," 2001). This definition is very wide and includes probiotic products with a large variability of expected effects on health. Following a recent Expert Consensus (Hill et al., 2014), the term "probiotic" includes three main categories: probiotics in food or supplements without health claim, probiotics in food or supplements with a specific health claim and probiotic drugs. If probiotics without health claims do not need specific strain evidence of efficacy and could benefit from extrapolated evidence based on taxonomical or functional comparisons, probiotics with specific health claims and probiotic drugs need appropriate evidences to support their use. For a specific health claim, data could derive from randomised clinical trials (RCTs) but also from large observational studies, whereas for probiotic drugs appropriate RCTs using the defined probiotic strain under consideration are required to meet regulatory standards for drugs. Probiotics with and without health claim are usually chosen by consumers directly, without physician suggestions, as they have healthy individuals as target population. Differently, the choice of a probiotic drug should be based on physician prescription as it includes a risk-benefit evaluation to justify its use in patients with a specific disease. However, only few probiotic products on the market meet all requirements to be considered a probiotic drug and, particularly, multistrain products are assembled based on the supposed synergistic effects of included strains, but these effects are often not evaluated in humans in RCTs, omitting that different strains could also display an antagonistic effect. Despite this limit, the choice of

Abbreviations: AAD, antibiotic-associated diarrhoea; CDAD, *C. difficile*-associated diarrhoea; CFU, colony forming unit; FMT, faecal microbial transplantation; HE, hepatic encephalopathy; hs-CRP, high sensitive C-reactive protein; IBS, irritable bowel syndrome; IBS-SSS, Irritable Bowel Syndrome -Symptom Severity Score; N, number; NAFLD, non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; qPCR, quantitative real-time polymerase chain reaction; RCTs, randomised, controlled, clinical trials; SCFA, short-chain fatty acids

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multistrain products is appealing for physicians, due to the supposed higher and pleiotropic efficacy compared to single strain products. In fact, beyond the theoretical synergistic effects among different strains, multistrain products usually contain higher total concentration of bacteria compared to monostrain products (Toscano, De Grandi, Pastorelli, Vecchi, & Drago, 2017), with a possible higher dose-related effect. However, direct comparisons of multistrain and monostrain probiotics are lacking in the literature and comparison among different multistrain products is difficult due to heterogeneity of data on the literature. Thus, the choice of the right probiotic could be challenging for Physicians. This review will focus on multistrain probiotic drugs, reviewing data from RCTs and meta-analysis of RCTs in gastrointestinal diseases, to provide an evidence-based guide to Physicians in the choice of multistrain probiotics.

A search of the Pubmed database was performed using the terms "multistrain probiotic", "mixed-species probiotic", "probiotic mixture", "probiotic interaction". References of relevant reviews or guidelines were manually checked.

As there is not a common validated definition of "multistrain" probiotics, we decided to consider "multistrain" products containing at least two strains of different probiotic species that belong to one or more genera (Timmerman, Koning, Mulder, Rombouts, & Beynen, 2004). Based on available product on the market, we decided to not exclude multistrain preparations also containing prebiotics (symbiotics) (de Vrese & Schrezenmeir, 2008). Only randomised, double-blind, placebo-controlled trials and meta-analysis of such RCTs were included. Randomisation is intended 1:1 when not otherwise specified. We selected trials on adult population. Open-label, single-blind or uncontrolled clinical studies were excluded. When available, results about microbiota evaluation before and after probiotic therapy have been reported.

Among the retrieved multistrain probiotic preparations, we found that *L. acidophilus* and *L. rhamnosus* are the most represented species (Fig. 1). The main fields of application of multistrain probiotic preparations are irritable bowel syndrome, *H. Pylori* eradication and antibiotic-associated diarrhoea (Table 1).

2. *Lactobacillus rhamnosus* GG (ATCC 53103), *Lactobacillus rhamnosus* LC705 (DSM 7061), *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM 7067), *Bifidobacterium breve* Bb99 (DSM 13692)

This four-strain probiotic mixture produced by Valio Ltd (Finland) was assembled as capsules containing a total amount of 8–9 × 10^9 colony forming unit (CFU) per capsule with an equal amount of each strain (Lyra et al., 2010) and even as a milk-based drink, distinguished by a total concentration of lactobacilli of 6 × 10^8 CFU/mL, *P. freudenreichii* ssp. *shermanii* JS of 7 × 10^8 CFU/mL and *B. breve* 7 × 10^6 CFU/mL.

2.1. Irritable bowel syndrome

This multistrain probiotic was first studied in 2005 in 103 patients affected by Irritable Bowel Syndrome (IBS) (Kajander, Hatakka, Poussa, Farkkila, & Korpela, 2005) in a 6-month RCT. Patients were randomised to one probiotic capsule daily or to placebo. Primary symptoms studied were: abdominal pain, distension, post-meal distension, distension following extended periods of sitting, flatulence and borborygmi. Whereas secondary symptoms were: urgency, feeling of incomplete evacuation, straining, belching, heartburn, nausea, post-meal nausea, post-meal fullness, vomiting and mucus or blood in stools.

The study population reported a global reduction in IBS symptoms after treatment, as the total symptom score (abdominal pain + distension + flatulence + borborygmi) was 7.7 (95% CI: −13.9 to −1.6) points lower in the probiotic group (p = 0.015) compared with placebo, with a median reduction of 42% in the symptom score in the probiotic group compared with 6% in the placebo group. Regarding individual symptoms, there were no differences between the two groups with the exception of borborygmi (p = 0.008), whereas for the rest of symptoms there was a non-significant trend toward reduction in the active group (see Table 2).

A subgroup of 42 patients participating in this RCT provided faecal samples before and 3 and 6 months after treatment for microbiome evaluation using quantitative real-time polymerase chain reaction (qPCR). The probiotic group (n = 22) showed a decrease in a phylotype with 94% similarity to *Ruminococcus torques* compared to placebo group (p = 0.02 at 6 months) and stably elevated levels of the clostridial phylotype, *Clostridium thermosuccinogenes* 85%, during the treatment (p = 0.00 and p = 0.02 at 3 and 6 months, respectively). These bacterial alteration correlated with the alleviation of IBS symptoms (Lyra et al., 2010).

Additional results arose in 2007 in a study involving 55 IBS patients fulfilling the Rome I or II criteria (Kajander et al., 2007), focusing on the microbiological effects detected by molecular and biochemical methods. During the 6-month study period, each subject received daily either a probiotic or a placebo capsule (28 and 27 patients, respectively). At the end of the treatment, 43 subjects completed the trial according to the protocol. During the study, faecal samples were collected from each subject at baseline, at 3-months and at 6-months and immediately frozen and stored until analysis. The quantification of ingested strains and selected bacterial species was performed by qPCR; strain-specific real-time PCR assays were developed for the quantification of *L. rhamnosus* GG, *L. rhamnosus* Lc705, *P. freudenreichii* ssp. *shermanii* JS and *B. breve* Bb99. In addition, indicators of microbial activity, such as faecal short-chain fatty acids (SCFA) content and bacterial enzymes, were determined. The prevalence rates in the probiotic group at 3- and 6-months were 89% (mean of log10 counts 7.0; 95% CI: 6.33–7.66) and 95% (6.7; 95% CI: 6.05–7.32) for *L. rhamnosus* GG and 89% (6.9; 95% CI: 6.38–7.42) and 79% (6.3; 95% CI: 5.69–6.97) for *B. breve* Bb99. *L. rhamnosus* Lc705 was found in 53% of subjects at both 3 and 6 months, whereas *P. freudenreichii* ssp. *shermanii* JS was detected in 84% (7.4; 95% CI: 6.49–8.39) at 3 months and in 63% at 6 months. On the other hand, in the placebo group *L. rhamnosus* GG was found in 43% at 3 months and in 24% at 6 months; the number of carriers of the other ingested probiotic strains was low in this arm, as *L. rhamnosus* Lc705 and *B. breve* Bb99 could not be detected in any samples at 3 or 6 months, while *P. freudenreichii* ssp. *shermanii* JS was found in the 10% of the samples at both 3 and 6 months. Intestinal microbiota remained stable during the trial, except for *Bifidobacterium* spp., which increased in the placebo and decreased in the probiotic group (p = 0.028). No changes in SCFA occurred. A decrease in ß-glucuronidase activity was detected in 67% of the subjects in the probiotic group vs. 38% in the placebo group (p = 0.06).

2.2. H. pylori eradication

A similar probiotic mixture, presented as a milk-based drink and containing a total of 1 × 10^9 CFU/mL, was then tested on a clinical trial focused on efficacy and tolerability of *H. pylori* eradication treatment with 7-day triple therapy with lansoprazole (30 mg), clarithromycin (500 mg) and amoxicillin (1 g) twice daily (Myllyluoma et al., 2005). Forty-seven patients were randomised to receive probiotics or placebo twice a day during *H. pylori* eradication therapy and once a day for 3 weeks following the treatment. Faecal samples were provided before the intervention (day 0), at the end of the eradication treatment (day 7), after the probiotic intervention (day 28) and after the 6-week follow-up, in order to assess the concentration of *L. rhamnosus* GG and *P. freudenreichii* ssp. *shermanii* JS. The primary outcome was the occurrence of new or aggravated symptoms during the eradication week compared to baseline and did not show any difference between the two groups. However, the probiotic arm reported improved tolerance to the eradication treatment when total symptom severity was considered (p = 0.038). Considering faecal samples, the mean faecal concentration