SUPPLEMENT ARTICLE

The Safety of Probiotics

David R. Snydman

Division of Geographic Medicine and Infectious Diseases and Department of Medicine, Tufts–New England Medical Center, and Tufts University School of Medicine, Boston, Massachusetts

Probiotics are generally defined as microorganisms that, when consumed, generally confer a health benefit on humans. There is considerable interest in probiotics for a variety of medical conditions, and millions of people around the world consume probiotics daily for perceived health benefits. Lactobacilli, bifidobacteria, and lactococci have generally been regarded as safe. There are 3 theoretical concerns regarding the safety of probiotics: (1) the occurrence of disease, such as bacteremia or endocarditis; (2) toxic or metabolic effects on the gastrointestinal tract; and (3) the transfer of antibiotic resistance in the gastrointestinal flora. In this review, the evidence for safety of the use of or the study of probiotics is examined. Although there are rare cases of bacteremia or fungemia related to the use of probiotics, epidemiologic evidence suggests no population increase in risk on the basis of usage data. There have been many controlled clinical trials on the use of probiotics that demonstrate safe use. The use of probiotics in clinical trials should be accompanied by the use of a data-safety monitoring board and by knowledge of the antimicrobial susceptibilities of the organism used.

Lactobacilli have a long history of safe use in foods and dairy products [1]. There is a natural association of lactobacilli with human flora, and lactobacilli are found in animals as well as plants [2]. Lactic acid bacteria have traditionally been used in fermented milks and by different societies around the world for the treatment of intestinal disturbances, especially in children [3]. Rarely, lactic acid bacilli will cause infection in humans, which has manifested as either bacteremia or endocarditis, particularly in immunocompromised hosts [4–9].

Lactobacilli fall into the category of organisms classified as "generally regarded as safe" [10]. Organisms that are generally regarded as safe include lactobacilli, lactococci, *Bifidobacterium,* and yeast. There are other probiotic organisms, such as *Enterococcus, Bacillus,* and other spore-forming bacteria, as well as streptococci, that are not generally regarded as safe but have been used as probiotics. In this review, I will focus on the data regarding the safety of probiotics. In addition, I will pay particular attention to the safety of *Lactobacillus rhamnosis* GG (*Lactobacillus* GG), given that this is the organism for which the most extensive number of human studies have been published [11–15]. It is also the organism that our group is currently pursuing in a series of research studies [16].

Table 1 provides the list of human populations in which *Lactobacillus* GG has been studied and in whom there is evidence of safety [11–15, 17–24]. The populations studied include pregnant women, premature neonates, elderly individuals, children with rotavirus diarrhea, children and adults hospitalized with diarrhea, malnourished children from Peru, patients with rheumatoid arthritis, adults with Crohn's disease, adults with *Helicobacter pylori* infection, and adults with *Clostridium difficile*–associated diarrhea. There are also a number of studies in which the safe use of other probiotics has been studied [25–30] (table 2). One subject of these studies has been the use of *Lactobacillus casei* Shirota to treat critically ill children. There are a number of studies of adults with *C. difficile*–associated diarrhea and the use of probiotics. The organisms studied in this context include *Lactobacillus plantarum, Saccharomyces boulardii,* and *Lactobacillus acidophilus* plus

Reprints or correspondence: Dr. David R. Snydman, Box 238, Tufts–New England Medical Center, 750 Washington St., Boston, MA 02111 (DSnydman@tufts-nemc.org).

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Table 1. Populations in whom *Lactobacillus* GG has been studied and has shown evidence of safety.

Pregnant women
Premature neonates
Elderly individuals
Children with rotavirus diarrhea
Hospitalized children
Hospitalized adults
Finnish and other tourists
Malnourished Peruvian children
Patients with rheumatoid arthritis
Adults with Crohn's disease
Adults with *Helicobacter pylori* infection
Adults with *Clostridium difficile*–associated diarrhea

Bifidobacterium. Studies have been performed in patients with Crohn's disease, employing a wide array of agents, including *Lactobacillus johnsonii* LA1 and VSL#3 (VSL Pharmaceuticals). There have been a large number of studies of the prevention and treatment of urinary tract infections in adult women, as well as of children attending day care, in whom the occurrence of both respiratory illness and diarrhea has been examined [31–35]. *L. plantarum* 299V has been studied in liver transplant recipients, adults in the intensive care unit, and adults with liver failure or chronic liver disease [36–38]. There are a number of studies of treatment of rotavirus diarrhea, including treatment with *Bifidobacterium lactis* (BB-12), *Lactobacillus reuteri* SD 2222, and many other probiotics [39, 40]. *S. boulardii* has been studied in patients with HIV-associated diarrhea and in adults with diarrhea and antibiotic-associated diarrhea [41, 42]. Intervention with probiotics in the treatment of bacterial vaginosis and vaginal candidiasis has also been well studied, with no significant adverse events; probiotics studied for this purpose include *Lactobacillus fermentum* (RC-14), *L. rhamnosis* GR-1, and *L. plantarum* [43, 44]. Many agents have been studied in patients with *H. pylori* infections, as well as in patients with irritable bowel syndrome [45–47].

THEORETICAL ADVERSE RISKS OF PROBIOTICS

There are some theoretical adverse risks that have been raised with respect to the use of probiotics in humans [2, 3, 48–52]. These theoretical risks include the potential for transmigration and the fact that colonization with probiotics may have a negative impact on gastrointestinal physiology and function, including metabolic and physiologic effects [1, 3, 49]. There could also be adverse immunologic effects, both localized and generalized [1, 50]. Finally, there is also the potential for antibiotic-resistance transfer within the gastrointestinal tract from commensal or probiotic bacteria to other bacteria or potential pathogens [3, 53].

Transmigration potential. With respect to potential toxicity due to transmigration, there is no evidence that probiotics have more adhesive properties than do clinical strains [10, 54]. There are a number of studies in animal models that demonstrate that there is no increase in the translocation of other bacteria when probiotics are given [55]. In addition, probiotics mitigate the transmigration of pathogens during their use [56]. There are some human studies showing that patients who are taking probiotics are actually less likely to have transmigration than are those who are not [56]. Animal evidence suggests that there is actually a reduction in the translocation of other bacteria, as opposed to the transmigration of probiotic bacteria into the bloodstream. There is no evidence, from population-based studies, of any increased risk of bacteremia or endocarditis due to probiotics [57]. There is also no evidence of any negative impact on the permeability of gut proteins in studies performed both in animals and in humans [58].

Bacteremia and endocarditis potential. We do know that lactic acid bacteria, including bifidobacteria, have been isolated as causes of bacteremia and also as causes of endocarditis [5–8, 49, 59, 60]. The list of organisms that have been associated with endocarditis or bacteremia includes *L. rhamnosis, L. plantarum, L. casei, Lactobacillus paracasei, Lactobacillus salivarius,*

Table 2. Populations in whom safe use of other probiotics has been studied.

Critically ill children (*Lactobacillus casei* Shirota)
Patients with *Clostridium difficile*–associated diarrhea (*Lactobacillus plantarum, Saccharomyces boulardii,* and *Lactobacillus acidophilus* plus *Bifidobacterium*)
Patients with Crohn's disease (*Lactobacillus johnsonii* LA 1, VSL#3)
Adult women with urinary tract infections
Children attending day care
Liver transplant recipients (*L. plantarum* 299V)
Adults in the intensive care unit (*L. plantarum* 299 V)
Patients with liver failure (*L. plantarum* 299 V)
Patients with rotavirus diarrhea (*Bifidobacterium lactis* BB-12, *Lactobacillus reuteri* SD 2222, and many others)
Patients with necrotizing enterocolitis (*L. acidophilus, Bifidobacterium infantis*)
Patients with HIV infection–associated diarrhea *(S. boulardii)*
Adults with diarrhea *(S. boulardii, L. casei, Streptococcus thermophilus, Bacillus bulgaricus, L. acidophilus)*
Adults with antibiotic-associated diarrhea *(L. plantarum, S. boulardii, L. acidophilus, B. bulgaricus)*
Patients with bacterial vaginosis and candida vaginitis (*Lactobacillus fermentum* RC-14 plus *Lactobacillus rhamnosus* GR-1, *L. plantarum*)
Patients with *Helicobacter pylori* infection (many)
Patients with irritable bowel syndrome (many)

L. acidophilus, and many other lactobacilli [5]. In addition, *Lactococcus lactis* and *Leuconostoc* species, as well as *Pediococcus* species have been demonstrated to cause bacteremia and endocarditis. *Bifidobacterium* species have also been isolated from the blood and in patients with endocarditis [61]. *Enterococcus* species, of course, are well known as causes of bacteremia and endocarditis [62].

With respect to sepsis related to probiotics, there have been 3 reports of *Lactobacillus* GG–associated bacteremia in children with short gut syndrome, 2 cases of bacteremia in children who have central venous catheters, 1 case of endocarditis, and 1 case of a liver abscess [6, 7, 60, 63, 64]. In addition, there has been a case of endocarditis caused by a strain of *L. rhamnosis* whose subspecies could not be completely specified. There have been 5 cases of bacteremia associated with *Bacillus subtlis* [59]. There has also been a case of *L. acidophilus* bacteremia in a patient who had HIV infection and Hodgkin disease [9] and a case of *Lactobacillus* infection after a bone marrow transplant [7].

Among the cases of *Lactobacillus* GG bacteremia in patients with short gut syndrome, 4 occurred in 3 separate events [6, 8, 58]. All of the cases were characterized by the presence of central venous catheters and intestinal feeding tubes. Two of the isolates were verified by PFGE as being *Lactobacillus* GG, and 1 was verified by both PFGE and PCR as being *Lactobacillus* GG. One of the isolates was not specifically verified as being *Lactobacillus* GG. Two of the 4 cases involved central venous catheter infections, and 2 had positive catheter culture results. These reports underscore the possible risk of *Lactobacillus* GG bacteremia related to the short gut syndrome. The source of the organisms might have been contamination of central venous catheters during manipulation, especially during feeding.

Data from surveillance in Finland suggest that there was no increase in *Lactobacillus* bacteremia during the decade 1990–2000 [65]. Lactobacilli represented 0.02% of all positive blood cultures. There was no temporal change over the decade. Another study from the National Public Health laboratory demonstrated that lactobacilli were present in 0.24% of positive blood cultures referred to the laboratory [66]. Although these cultures were reported to have lactobacilli, 27% could not be confirmed. *Lactobacillus* GG accounted for 11 of the 26 *L. rhamnosis* strains that were recovered from the blood. *L. rhamnosis* constituted 54% of all the lactobacilli that were isolated. The absence of any change in the prevalence of *Lactobacillus* bacteremia and, specifically, the absence of a change in *Lactobacillus* GG bacteremia is remarkable, given that the consumption of *Lactobacillus* GG increased in Finland from 1 L per person per year to 6 L per person per year over the period studied [65].

Of the 89 cases of *Lactobacillus* bacteremia in Finland from 1990 to 2000, 53% had species identification [66]; 25 had *L. rhamnosis* confirmed, and 22 had other lactobacilli. Eleven cases were indistinguishable from *Lactobacillus* GG by PFGE. None of these cases was associated with endocarditis. Most of the patients had serious comorbidities. Appropriate therapy was shown to improve survival [66]. Mortality appeared to be associated with the severity of underlying illness.

Lactobacillus bacteremia in Sweden was examined over a 6-year period, during which time there was an introduction of 3 probiotic strains into clinical use [67]. The probiotics studied were *L. paracasei paracasei, L. acidophilus* NCFB 1478, and *Lactobacillus* GG. There was no change in the rate of lactobacillemia, and no case in which *Lactobacillus* was isolated from the blood stream was identified as being related to the probiotic strains. The authors of the study recognized that most cases of lactic acid bacteremia are actually polymicrobial.

There have, however, been cases of sepsis related to probiotics. The most prominent have been associated with *S. boulardii* [68–72]. There have been 16 reports of candidemia, encompassing 23 patients. Some of these patients developed septic shock. Many of the cases had some degree of molecular identification and confirmation of the probiotic strain [73, 74].

Gastrointestinal toxicity studies. With respect to the potential impact of the use of probiotics on gastrointestinal physiology, there is the possible production of metabolites that might be undesirable, especially in patients with short small bowel syndrome [75]. There is a theoretical risk that the probiotic bacteria might lead to malabsorption due to deconjugation of bile salts [76]. This might, therefore, increase the risk of colon cancer [77]. However, there is no epidemiologic or clinical evidence to support this hypothesis [78], and there are experimental data to demonstrate some inhibitory effect of probiotics for colon cancer in animal models [79, 80].

Among the additional potential toxicities, there is also a theoretical possibility that D-lactate production might occur, with the development of lactic acidosis [81]. Studies have been performed in healthy humans with an ileostomy. *L. acidophilus* and *Bifidobacterium* species have been shown to transform conjugated bile acid into nontoxic secondary salts [81]. In patients with short small bowel syndrome, it is possible that the conjugated bile acid metabolites might accumulate and lead to malabsorption [82]. This might lead to the risk of the lactate accumulation and a theoretical risk of colon cancer. There is also the theoretical possibility that there may be degradation of intestinal mucus [83]. However, in studies both in vitro and in gnotobiotic rats, there is no evidence that probiotics will degrade intestinal mucus [50, 84].

Studies suggest that probiotics may modulate the immune response of individuals and boost response to vaccines or alter the natural history of the allergic response. Probiotic bacteria can modify humoral, cellular, and nonspecific immune responses and may have an impact on the local secretion of cytokines as well as the local immune response [3]. It is thought that some of these responses are strain specific and host specific [3]. The role of intestinal microflora in immune development suggests that a theoretical possibility exists that manipulations caused by probiotics could have an adverse immunomodulatory effect. An additional population in which a theoretically adverse immunologic impact might be postulated is pregnancy. However, the use of probiotics during pregnancy, in neonates, and in children has not been associated with any adverse immunologic effects [18–21, 23–25, 30, 51, 52, 85].

Antibiotic-resistance transfer. A major area of concern has been the potential for antibiotic-resistance transfer in the gastrointestinal tract that might take place between probiotics and pathogenic bacteria [53, 86]. When one examines the potential for transferable antibiotic resistance in lactic acid bacteria, one can find the presence of plasmids with antibiotic-resistance genes, including genes encoding resistance to tetracycline, erythromycin, chloramphenicol, and macrolide-lincosamide-streptogramin [87]. These resistance plasmids have been found in *L. reuteri, L. fermentum, L. acidophilus,* and *L. plantarum* in raw meat, silage, and feces of animals [88]. Streptomycin resistance, tetracycline resistance, and chloramphenicol resistance, as well the plasmid mef 214, have been found in *L. lactis* in raw milk and soft cheese. Tetracycline resistance has been found in *L. plantarum* 5057 [89].

The transfer of native *Lactobacillus* plasmids is quite rare. Lactose fermentation plasmids have been transferred to *L. casei* [90]. Bacteriocin production has been transferred to *L. johnsonii.* There is some evidence that *Leuconostoc* species and *Pediococcus* species can accept broad-host-range antibiotic-resistance plasmids from *Lactococcus* species [91]. Conjugation transfer from enterococci to lactobacilli and lactococci can occur in the gut of animals, as well as in vitro; however, the transfer to lactobacilli is quite rare [86, 92]. There have been some attempts to transfer antibiotic resistance with a broad-host-range plasmid pAMB. Of 14 strains of *Lactobacillus delbrueckii,* 44 strains of *L. acidophilus,* 1 strain of *Lactobacillus helveticus,* 1 strain of *Lactobacillus brevis,* 6 strains of *L. casei rhamnosis,* 5 strains of *L. plantarum,* and 1 strain of *L. fermentum,* only 1 strain each of *L. helveticus* and *L. brevis* accepted the plasmid with low efficiency (10^{-7}) [93]. A tetracycline-resistance determinant has been found in *Lactobacillus* organisms isolated from dried sausages. Seven of 14 strains were able to transfer resistance from *Lactobacillus* to *Enterococcus* at frequencies of 10^{-4}–10^{-7} [86, 94]. Two of 14 strains could transfer to *L. lactis* but were unable to transfer to *Staphylococcus aureus* [94].

There have also been attempts at molecular identification of vancomycin-resistance genes in lactobacilli. Five strains of *L. reuteri* and 1 strain of *L. rhamnosis* were probed for *vanA, vanB,* and *vanC* genes. None were found [95]. *Lactobacillus* GG has been studied specifically, and no plasmids have been found; there is no evidence of *vanA, vanB, vanH, vanX, vanZ, vanY,* and *vanS,* by hybridization or PCR [96].

THE SAFETY OF *LACTOBACILLUS* GG

Lactobacillus GG has been the given to several thousands of individuals in clinical trials [11–15, 17–24]. It has been administered to travelers with diarrhea in Mexico, as well as to travelers to Turkey. It has been administered to children with chronic inflammatory disease, including Crohn's disease and juvenile rheumatoid arthritis, to adults with inflammatory bowel disease, and to patients with HIV infection [97]. It has also been administered to children and pregnant women and adults with multiple food allergies. To date, no significant adverse events have been demonstrated in these and other controlled trials [16].

There are a number of intrinsic properties that are a testament to the safety of *Lactobacillus* GG, including the absence of any plasmids. There appear to be no plasmids that contain transferable or other antibiotic resistance. The vancomycin resistance that has been found appears to be nontransferable and chromosomal [97]. The organism has a good enzyme profile. It elaborates β-glucoronidase and urease, and it also secretes an antimicrobial agent [98, 99]. It appears to prevent attachment or invasion of pathogens in cell culture systems in vitro [100]. It has also not been associated with platelet aggregation [101]. There is no breakdown of human intestinal glycoprotein or hog gastric mucin in vitro [102]. There has been no demonstration of mucus degradation in germ-free animals [103]. In addition, there is no invasion of Caco-2 or HeLa cell cultures, and there is evidence of prevention of pathogen invasion in cell culture systems [98].

There is no acute toxicity in mice, and, in fact, one cannot achieve a lethal oral dose in a mouse [104]. It has been given orally to lethally irradiated mice and actually prolongs survival [104, 105]. It does not translocate to either spleen or lymph nodes. It also inhibits tumor formation and binds aflotoxin [106, 107]. It has been administered to well more than 3000 healthy volunteers [16, 65, 103, 104]. There is also some evidence of phenotypic differences between commercial *Lactobacillus* GG and *L. rhamnosis* isolated from blood [108]. In these studies, it appears that *Lactobacillus* GG has decreased in vitro adhesion and has greater resistance to serum-mediated killing. It also induces a respiratory burst [108].

In conclusion, *Lactobacillus* GG has been proven safe both in vitro and in vivo (in animal models), as well as in a number of human studies [16, 65]. Although there have been rare cases of bacteremia and liver abscess in patients with short gut syndrome, overall, it is a safe probiotic. There is no other probiotic that has undergone extensive safety evaluation to a degree comparable to that undergone by *Lactobacillus* GG.

GENETICALLY ENGINEERED PROBIOTICS

Genetic modification of probiotics has been undertaken to increase certain physiologic or immunologic properties within the organism and to use the probiotic as a mucosal delivery system or a vaccine vector [109]. The use of these genetically engineered products has been quite limited to date, but the steps enumerated below should be taken for the use of any engineered strains introduced into human studies. As with any genetically engineered product, some caution must be employed when assessing safety.

STEPS TO MONITOR SAFETY OF PROBIOTICS

To monitor the safety of probiotics as they are introduced and increasingly used around the world, it is important to conduct population-based surveillance for the isolation of probiotic bacteria from patients with infection. There should be knowledge of the susceptibility profile for any strain used in clinical trials [110, 111]. There should be the ability to compare the clinically isolated strain with the probiotic strain by use of molecular methods. Any trial employing a probiotic strain should have active surveillance for cases of infection associated with such use and should have active surveillance for the occurrence of other adverse effects. Although some caution may be necessary in any trial of probiotics, concern about toxicity should not preclude their study. Rather, each study should be evaluated on a case-by-case basis, examining the risk benefit and potential toxicity. There is a list of patients for whom caution might be warranted, such as those with immune compromise, premature infants, those with short bowel syndrome, those with central venous catheters, elderly patients, and those with cardiac valve disease. However, the presence of any of these factors may not necessarily preclude a clinical trial. Each study should be evaluated on a study-by-study basis, with the appropriate involvement of a human investigation review committee and a data-safety monitoring committee, as well as specific hypotheses to be tested and surveillance for bloodstream infection with the probiotic strain. Ideally, there should be population-based surveillance for *Lactobacillus* bacteremia, including the use of a reference laboratory and molecular confirmation.

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