Are Probiotic Really Safe for Humans?

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Abstract

Probiotic bacteria have been used as a health-promoting factor for a very long time. Nowadays, products containing probiotic bacteria are becoming more and more popular on the market. The term probiotics refers to the products belonging to the following groups: probiotic drugs (medicinal products – live biotherapeutic products for human use), medical devices, probiotic foods (e.g. foods, food ingredients, dietary supplements or food for special medical purposes), directly fed microorganisms (for animal use) and designer probiotics (genetically modified probiotics). Safety assessment of bacterial strains used as probiotics should be carefully studied. Even though probiotic bacteria have the generally recognized as safe (GRAS status), there are several reports about side effects triggered by the presence of these organisms. Microorganisms used as probiotics may cause systemic infections, stimulate the immune system, disturb metabolism and participate in horizontal gene transfer.

Key words: bacteremia, gene transfer, probiotic bacteria, probiotic side effect, safety of probiotics

Introduction

The probiotics are live microorganisms that, when administrated in adequate doses, confer a health benefit to the host as defined by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO 2001). For this purpose, lactic acid bacteria (LAB) have been used for a very long time. At the beginning of the twentieth century, Ilja Metchnikoff suggested that the longevity of Bulgarians is due to consumption of fermented milk products (Metchnikoff 1908). Another scientist, Henry Tissier observed that in stools from children with diarrhea, only a small number of bacteria with Y-shaped morphology was present, while in healthy children, a large amount was observed (Tissier 1906). In 1965, Lilly and Stillwell used term "probiotic" to describe substances that were secreted by one organism and stimulated the growth of another (Lilly and Stillwell 1965).

Probiotic properties have been observed in many genera of bacteria and fungi, but the most commonly used probiotics belong to the species of *Lactobacillus* and *Bifidobacterium*. In addition, other bacteria genera,

like Streptococcus, Enterococcus, and Bacillus, as well as members of the yeast genus Saccharomyces can have probiotic properties (Hempel et al. 2011). The most common species include: Lactobacillus acidophilus, Lactobacillus johnsonii, Lactobacillus gasseri, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus plantarum, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium bifidum and Bifidobacterium infantis (Ishibashi and Yamazaki 2001). Some bacteria, not regularly present in the gastrointestinal tract, like Lactobacillus bulgaricus, Streptococcus thermophilus, Leuconostoc and Lactococcus species may also belong to the category of probiotic microorganisms and are usually used as starters in dairy products (Ishibashi and Yamazaki 2001). Most probiotic species, including lactobacilli, Bifidobacterium, lactococci, and some yeasts, are classified as the "generally recognized as safe" (GRAS). But there are groups of organisms, like streptococci, enterococci, Bacillus and other spore-forming bacteria, that do not possess GRAS status but have been used as probiotics (Snydman 2008). It should be noticed that not all bacteria of a given genus or species have probiotic features, they are assigned only to specific

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strains (Hill et al. 2014). The origin of the strain, resistance to antibiotics, as well as the lack of pathogenicity determines the safety of probiotic strains (Markowiak and Śliżewska 2017).

The term "probiotic" can refer to the following products: probiotic drugs (medicinal products - live biotherapeutics products for human use), medical devices, probiotic foods (e.g. foods, food ingredients, dietary supplement or food for special medical purposes), directly fed microorganisms (for animal use) and genetically modified probiotics (Venugopalan et al. 2010). Probiotics have an annual market growth of 7% globally (Johnson and Klaenhammer 2014; McFarland 2015) and are projected to grow to \$65 billion in 2024 (Global Market Insights 2017). Guidance is required to assess the safety of probiotics, but several issues should be taken into account before, i.e., a large variety of probiotic strains, the risks associated with the use of unclassified strains, and the possibility of unsurpassed interactions between both the strains used and the host and bacteria (Gueimonde et al. 2004). Probiotics may be responsible for systemic infections (Marteau 2001; Doron and Snydman 2015), excessive immune stimulation in susceptible individuals (Marteau 2001; Borriello et al. 2003; Doron and Snydman 2015), deleterious metabolic effects (Marteau 2001; Doron and Snydman 2015) or gene transfer (Marteau 2001; Ashraf and Shah 2011; Doron and Snydman 2015). The factors that must be considered in assessing the safety of probiotic products should include infectivity, pathogenicity, an excessive immune stimulation in susceptible individuals, virulence factors comprising toxicity, metabolic activity and the important properties of microbes (Ishibashi and Yamazaki 2001). The Lactic Acid Bacteria Industrial Platform has submitted reports from the European Union indicating that except for enterococci the risk of infections caused by lactic acid bacteria is very low (Liong 2008).

Infections

The *Lactobacillus* strains are present in healthy humans in the oral cavity, ileum and colon; moreover, they are the main microorganisms in the vagina (Borrielo et al. 2003). The *Lactobacillus* and *Bifidobacterium* strains as commensals of human microbiome are safe and nonpathogenic. There is no evidence that consumption of probiotic lactobacilli or bifidobacterial strains poses the risk of infection greater than that related to commensal strains. The number of cases of infection caused by *Lactobacillus* or *Bifidobacterium* strains are very low and vary between 0.05–0.4% for infective endocarditis and bacteremia (Borrielo et al. 2003). The translocation of bacteria may be caused by

a weakened intestinal barrier, resulting in the passage of bacteria across the mucus membrane and epithelium (Berg 1985). The next step is transport of microorganisms through the tunica propria to the mesenteric lymph nodes and other organs (Ishibashi et al. 2001). This translocation may result in bacteremia, followed by multiple organ failure and septicemia (Berg 1992; Van Leeuwen et al. 1994). Various factors such as damage of the mucous membrane, disturbances in the composition of the intestinal microflora, or diminishing of the host's immune system may stimulate the translocation of the intestinal bacteria (Ishibashi et al. 2001). In some clinical reports, probiotic bacteria have been identified as casual factors of dental cavities, endometritis (Bayer et al. 1978), urinary tract infections (Brumfitt et al. 1981; Dickgieber et al. 1984), meningitis (Sharpe et al. 1973) and spleen abscesses (Sherman et al. 1981). These infections can be associated with recent surgery, organ transplant, AIDS (Patel et al. 1994; Horwitch et al. 1995; Land et al. 2005; Ambesh et al. 2017), valvopathy, diabetes mellitus, immunosuppressive cancer therapy or cancer with antibiotic treatment, which may contribute to selection of specific microorganisms (Liong et al. 2008). Increased consumption of products containing probiotic bacteria has not led to an increase of the aforementioned opportunistic infections in consumers (Borrielo et al. 2003). In an epidemiological study of Lactobacillus bacteremia frequency in Finland, no correlation was found between the increased use of L. rhamnosus GG and the occurrence of Lactobacillus bacteremia during 1990–2000 (Salminen et al. 2002).

The cases of children infections, including bacteremia, caused by the Lactobacillus strains are quite rare and have been observed mainly in immunocompromised children (Bayer et al. 1978; Kalima et al. 1996; Schlegel et al. 1998, Land et al. 2005), and in patients with neonatal sepsis and meningitis (Brughton et al. 1983; Thompson et al. 2001), pneumonia (Sriskandan et al. 1993), and also local suppurative infections (Brook 1996). Probiotic therapy administered to preterm infants and neonates should be considered carefully, since at birth infants do not have a fully developed immune system, and thereby after probiotic administration the risk of fungemia or bacteremia significantly increases (Boyle et al. 2006; Marodi 2006). Lactobacillus bacteremia among children is very unusual, however, there were reports on three infants with short-bowel syndrome that developed bacteremia after consumption of *L. rhamnosus* GG (Kunz et al. 2004; De Groote et al. 2005). Land et al. (2005) reported two pediatric patients who had ingested probiotic strain L. rhamnosus GG and subsequently developed bacteremia and sepsis due to infection with Lactobacillus species. Vahabnezhad et al. (2013) described a case of Lactobacillus bacteremia in a 17-year-old boy with ulcerative colitis after ingestion

of L. rhamnosus GG. The 16S rRNA genes similarity was 99.78% between the L. rhamnosus strain isolated from the patient's blood and the consumed probiotic strain. This information suggests that people with ulcerative colitis are potentially at the risk of bacteremia due to the Lactobacillus strains (Vahabnezhad et al. 2013). Analysis of the results of 74 different controlled clinical trials showed that the use of probiotic and symbiotic in children between 0 and 18 years old was not linked to an increased health risk (Van den Nieuwboer et al. 2015). L. rhamnosus and L. casei, which are commonly present in the commercial probiotic products, belong to the strains most frequently isolated during bacteremia caused by Lactobacillus bacteria (Salminen et al. 2004). These Lactobacillus species may have a greater potential to translocate and therefore, they could be more pathogenic than other species (Liong 2008). Other probiotic microorganisms directly related to bacteremia and endocarditis are: L. plantarum, L. paracasei, L. salivarius, L. acidophilus and many other lactobacilli. Moreover, Lactococcus lactis, Leuconostoc, Pediococcus and Bifidobacterium have also been shown to induce bacteremia and endocarditis (Snydman 2008). The studies by Harty and coworkers (Harty et al. 1993; Harty et al. 1994) showed that L. rhamnosus strains, isolated from patients with endocarditis, possess capability to aggregate platelets and to adhere to fibronectin, fibrinogen and collagen. This aggregation could be related to proteins of the intestinal epithelium. In addition, L. rhamnosus and L. paracasei subsp. paracasei produce enzymes that degrade human glycoproteins and fibrin clots, and this observation suggests that these molecules may participate in the development of infective endocarditis (Liong et al. 2008). Kochan et al. (2011) described a case of sepsis caused by L. rhamnosus in a woman with a heart valve. A bacterial translocation probably occurred by a leaky intestinal barrier and led to heart failure in this patient (Kochan et al. 2011). Some cases of Lactobacillus-related bacteremia have been reported, including L. rhamnosus GG, L. casei and L. acidophilus (De Groote et al. 2005; Ledoux et al. 2006; Vahabnezhad et al. 2013). Moreover, nine cases of sepsis have been described, associated with S. boulardii, L. rhamnosus GG, Bifidobacterium breve, Bacillus subtilis or combination of probiotic bacteria (Doron and Snydman 2015). Fungemia, caused by Saccharomyces cerevisiae var. boulardii is the most commonly reported single infection (33 reports) (Doron and Snydman 2015). Among 89 strains from blood samples, analyzed by pulse-field gel electrophoresis, eleven strains had identical PFGE patterns as the probiotic strain L. rhamnosus GG (Salminen et al. 2004). However, the other studies showed that pathogenic L. rhamnosus GG-like strains isolated from the blood cultures, were phenotypically different from probiotic L. rhamnosus

GG (Ouwehand et al. 2004). Cases of deaths of healthy people caused by the intake of probiotic bacteria are very rare. The percentage of lethal infections caused by *Lactobacillus* is very low; however, these bacteria may infrequently cause bacteremia or endocarditis (Doron and Snydman 2015).

The probiotic strains have different antimicrobial susceptibility. The high doses of penicillin or ampicillin with or without aminoglycosides are most often used in treatment of lactobacilli infections. The retrospective study of 45 cases of bacteremia, demonstrated that 100% of lactobacilli were susceptible to ampicillin, clindamycin and erythromycin, 96% were susceptible to penicillin and 67% to gentamycin (Sherid et al. 2016). On the other hand, bifidobacteria are generally susceptible to β -lactams, glycopeptides and erythromycin (Moubareck et al. 2005), while fungemias caused by *Saccharomyces* strain may be treated with fluconazole, amphotericin B or voriconazole (Burkhardt et al. 2005).

Stimulation of the immune system

Probiotic strains may modulate the immune response of individuals, and this may result in the increased response to vaccines or allergens. Besides, these strains also have an effect on both cellular and humoral responses, and affect the secretion of cytokines (Senok et al. 2005). Side effects, such as fever or arthritis, can be caused by the presence of peptide-glycan-polysaccharides, which are components of the bacterial cell wall, e.g. of the Lactobacillus genus (Marteau 2001). The immune system of healthy and immunocompromised individuals may react differently to probiotic bacteria. For example, probiotic bacteria may exert a stimulating effect on phagocytosis in healthy people, whereas for people with allergies, this effect can be opposite (Senok et al. 2005). The immunomodulatory effect can also depend on the dose of probiotic used (Tsai et al. 2012). Despite the lack of direct reports on the probiotics harmful for immunocompromised individuals, it seems important to continue research on the efficacy and safety of probiotics (Sanders et al. 2010; Doron and Snydman 2015).

Detrimental metabolic effect

Probiotic bacteria during colonization of the small bowel deconjugate and dehydroxylate bile salts what could results in diarrhea and intestinal lesions (Agostoni and Salvini 2009). Since probiotic strains can produce bile salt hydrolase (BSH), the deconjugated bile salts could be accumulated, and then altered into harmful secondary bile acids by intestinal microbiota.

The accumulation of these cytotoxic compounds in the enterohepatic circulation could increase the risk of cholestasis and colorectal cancer (Tan et al. 2007). The harmful effects exerted by BSH on humans have not yet been accurately described (Ooi and Liong 2010). The probiotic *L. acidophilus* and *Bifidobacterium* spp., the strains derived from fermented milk products, may also convert the primary bile salts to secondary bile salts. If decarboxylation and dehydroxylation processes occur in excess, then there may be a potential risk to the patient's health (Marteau et al. 1995).

Other deleterious metabolic effect is D-lactate production by probiotic strains (Doron and Snydman 2015). Humans produce the L(+)-isomer of lactic acid, while the presence of D(-)-lactate is the result of bacterial metabolism or transformation of L(+)-lactate by a bacterial DL-lactate racemase (Hove and Mortensen 1995; Sanders et al. 2010). Some of the Lactobacillus strains may produce L(+)-lactic acid as well as D(-)lactic acid and transform one isomer into the other (Vitetta et al. 2017). In healthy humans, the increased level of D-lactic acidosis is rarely observed (Vitetta et al. 2017), but in children with short bowel syndrome the blood concentration of D-lactic acid is high during an exacerbation of symptoms (Bongaerts 2000; Sanders et al. 2010). Five reports of D-lactic acidosis can be found in the literature (Doron and Snydman 2015), of which two concern the patients with short bowel syndrome, both proceeded by the administration of probiotics (Łukasik et al. 2018). Infants may be more vulnerable to D-lactic acidosis, because of weaken barrier function of the intestinal tract and reduced ability of renal excretion (Sanders et al. 2010; Łukasik et al. 2018). In patients at risk of developing D-lactic acidosis, especially those with former bowel surgery and subsequent gut syndrome, and in newborns and neonates, administration of probiotics which may produce D(-)-lactate should be handled very carefully (Sanders et al. 2010).

Probiotic deleterious metabolic activities, such as degradation of mucin may also contribute to potential side effects after their consumption. In this case, the number of microorganisms that translocate through the small bowel is increased, possibly causing gastrointestinal disturbances including intestinal inflammation. One hypothesis states that the accumulation of probiotics in the gastrointestinal tract could result in increased risk of intestinal mucus degradation, but it was not confirmed. Ruseler-van-Embeden et al. (1995) conducted experiments on germ-free rats. They studied the degradation of mucus glycoprotein by Lactobacillus casei strain GG, L. acidophilus, B. bifidum and lactic culture isolated from fermented products, but did not observe any degradation of intestinal mucus glycoproteins or damage of the intestinal mucus layer (Ruseler-van Embden et al. 1995). Moreover, Abe et al.

(2010) did not observe translocation, damage of epithelial cells or any changes of the mucosal layer in the ileum, cecum and colon.

Potential transfer of genes

Another aspect concerning the safety of bacteria used as probiotics is the potential transfer of the antibiotic resistance genes between probiotics and other commensal or pathogenic bacteria that occur in the gastrointestinal tract (Teuber et al. 1999; Salyers et al. 2004; Snydman 2008; Nawaz et al. 2011). It has been reported that over 68% of probiotic strains were resistant to two or more antibiotics. Moreover, Bacillus strains from some probiotic products display even a high-level resistance. Lactic acid bacteria are naturally resistant to some antibiotics (Gueimonde et al. 2004). Many lactobacilli strains, apart from L. delbrueckii subsp. bulgaricus, L. acidophilus, L. johnsonii and L. crispatus are naturally resistant to vancomycin (Charteris et al. 1998; Balletti et al. 2009; Liu et al. 2009; Shao et al. 2015; Guo et al. 2017); however, the genes responsible for this resistance are chromosomally located and not easily transferable to other genera (Tynkkynen et al. 1998; Shao et al. 2015). Lactobacillus species are often resistant to aminoglycosides, monobactams and fluoroquinolones (Zheng et al., 2017). Guo et al. (2017) reported that 70% of the Lactobacillus strains tested were resistant to ciprofloxacin. The resistance of LAB strains to tetracycline, erythromycin, chloramphenicol, streptomycin, lincosamides and streptogramins may be due to the presence of plasmids bearing the genes encoding resistance to these antibacterial agents (Doron and Snydman 2015). The most commonly found resistance genes in Lactobacillus species are *tet*(M) and *erm*(B), responsible for tetracycline and erythromycin resistance, respectively. Moreover, the cat gene encoding chloramphenicol acetyltransferase was also present in LAB (Fukao and Yajma 2012). The tet(M) genes localized in Lactobacillus strains of various species, derived from fermented food possess a high similarity to the genes present in the pathogenic meningococcal or Staphylococcus aureus strains (Gevers et al. 2003a; Gevers et al. 2003b). The resistance plasmids carrying the *tet*(M) and *erm*(B) genes have been found in L. reuteri, L. fermentum, L. acidophilus and L. plantarum isolated from raw meat, silage and feces of animals (Snydman 2008). The isolates of *Leuconostoc* and Pediococcus can accept broad host range antibiotic resistance plasmids from Lactococcus species (Dessart and Steenson 1991). Moreover, in vitro conjugation transfer may occur from Enterococcus to Lactococcus and Lactobacillus (Doron and Snydman 2015). In some Lactobacillus strains (e.g., L. plantarum, L. reuteri, L. fermentum) a transmission of antibiotic resistance genes to other LAB strains occur via the pAMß plasmid (Schjørring and Krogfelt 2011). Analysis of gene transfer from 44 strains of L. acidophilus, 14 strains of L. delbrueckii, six strains of L. casei rhamnosus, five strains of L. plantarum, one strain of L. helveticus, one strain of L. brevis and one strain of L. fermentum, showed that only a single strain of *L. helveticus* and one *L. brevis* accepted the plasmid with low efficiency (Morelli et al. 1988). Sybesma et al. (2013) compared the genes from three bacterial isolates derived from dairy products containing the *L. rhamnosus* GG strains with a reference strain from the ATCC collection (L. rhamnosus 53103). In two of three strains tested, the lack of DNA fragments encoding 34 and 84 genes that affect the adhesion of these strains and their persistence in the gastrointestinal tract was observed (Sybesma et al. 2013). These examples underline the potential for genetic variation in LAB and other probiotic bacteria, and suggest that these changes may occur in the microbial cultures used for commercial processes (Sanders et al. 2014).

The risk of transfer of antibiotic resistance genes may be an argument against use of probiotic, for example in aquaculture. On the other hand, some antibiotic resistance phenotypes, for example resistance of few lactobacilli strains to vancomycin (Tynkkynen et al. 1998), or resistance represented by *Streptomyces* strains, are not easily transferable to other genera (Tan et al. 2016). Bacterial strains used as probiotic drugs, foods or dairy products should be systematically controlled in order to detect the antibiotic resistance genes that could be transferable to pathogenic bacteria. According to the guidelines of European Food Safety Authority (EFSA) all bacterial strains, used as feed additives, should be tested to determine their sensitivity to commonly used antibiotics (European Food Safety Authority 2012).

Identification

The correct identification of strains is extremely important for safety, growth conditions and metabolic properties of a given strain (FAO/WHO 2002). The correct classification of strains in a microbiome is extremely important at the time of preparation of the probiotic since this information is crucial for assessment of the stability of the strain, and to perform comparison with clinical isolates in the case of infection (Sanders et al. 2010). Even if the strain of probiotic bacteria is considered as safe, it still can cause bacteremia (Van den Nieuwboer et al. 2014) as opportunistic bacteria.

Microbiological examination of probiotic products showed that they might contain some microorganisms that were not indicated on the label (Hoa et al. 2000; Temmerman et al. 2002; Coeuret et al. 2004; Millazzo et al. 2004; Masco et al. 2005; Zawistowska-Rojek et al. 2016). The presence of these microorganisms indicates an inadequate control of production or insufficient control procedures. Some of the available probiotic products are labeled poorly, with lack of information not only on the classification of the strains included, but also about the excipients present in product, such as cow's milk protein, which may be an allergen for some people (Moneret-Vutrin et al. 2006; Lee et al. 2007).

Conclusion

The major risk factor in safe applications of probiotic microorganisms is the lack of knowledge on their activity. The interactions between intestinal microbes and the host have a major influence on the overall health condition. The suitable characteristics of relationships between probiotic structure and function would reduce the possibility of side effects. Generally, probiotic bacteria have a beneficial effect on the digestive system but in some cases they may facilitate the translocation or induce infections themselves. Due to the fact that the adverse effects caused by probiotics are documented, it is necessary to fully understand the mechanisms of activity of probiotic bacteria. In addition, the differences in activity of a single strain or a mixture of strains of different species or even genera should also be taken into account before the probiotic use in humans (Sanders et al. 2010).

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