2018, Vol. 67, No 3, 251–258
https://doi.org/10.21307/pjm-2018-044

Are Probiotic Really Safe for Humans?

ANNA ZAWISTOWSKA-ROJEK[1, 2]* and STEFAN TYSKI[1, 2]

[1] Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland
[2] Department of Pharmaceutical Microbiology, Medical University of Warsaw, Warsaw, Poland

Submitted 29 March 2018, revised 6 May 2018, accepted 14 June 2018

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

* Corresponding author: A. Zawistowska-Rojek, Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland; **e-mail: ania.zaw@gmail.com**

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. fer-*

mentum) a transmission of antibiotic resistance genes to other LAB strains occur via the pAMβ 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).

Literature

Abe F, Muto M, Yaeshima T, Iwatsuki K, Aihara H, Ohashi Y, Fujisawa T. 2010. Safety evaluation of probiotic bifidobacteria by analysis of mucin degradation activity and translocation ability. Anaerobe. 16:131–136.

Agostoni C, Salvini F. 2009. Probiotics in Infant Dietetics. In: Michail S, Sherman PM, editors. Probiotics in Pediatric Medicine. New York (USA): Humana Press.

Ambesh P, Stroud S, Franzova E, Gotesman J, Sharma K, Wolf L, Kamholz S. 2017. Recurrent *Lactobacillus* bacteremia in a patient with leukemia. J Investig Med High Impact Case Rep. 5:1–3.

Ammor MS, Florez AB, Mayo B. 2007. Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. Food Microbiol. 24:559–570.

Ashraf A, Shah NP. 2011. Antibiotic resistance of probiotic organisms and safety of probiotic dairy products. Int Food Res J. 18:837–853.

Bayer AS, Chow AW, Betts D, Guze LB. 1978. Lactobacillemia: report of 9 cases: important clinical and therapeutic considerations. Am J Med. 64:808–813.

Belletti N, Gatti M, Bottari B, Neviani E, Tabanelli G, Gardinni F. 2009. Antibiotic resistance of lactobacilli isolated from two Italian hard cheeses. J Food Prot. 72:2162–2169.

Berg RD. 1985. Bacterial translocation from the intesties. Exp Anim. 34:1–16.

Berg RD. 1992. Translocation and the indigenous gut flora. In: Fuller R. Probiotics: The Scientific Basis. London (UK): Chapman & Hall. p. 55–85.

Bongaerts G, Bakkeren J, Severijnen R, Sperl W, Willems H, Naber T, Wevers R, van Meurs A, Tolboom J. 2000. Lactobacilli and acidosis in children with short small bowel. J Pediatr Gastroenterol Nutr. 30:288–293.

Borriello SP, Hammes WP, Holzapfel W, Marteau P, Schhrezenmeir J, Vaara M, Valtonen V. 2003. Safety of probiotics that contain lactobacilli and bifidobacteria. Clin Infect Dis. 36:775–780.

Boyle RJ, Robins-Browne RM, Tang ML. 2006. Probiotic use in clinical practice: what are the risks? Am J Clin Nutr. 83:1256–1264.

Brook I. 1996. Isolation of non-sporing anaerobic rods from infections in children. J Med Microbiol. 45:21–26.

Brughton RA, Gruber WC, Haffar AA, Baker CJ. 1983. Neonatal meningitis due to *Lactobacillus*. Pediatr Infect Dis J. 2:382–384.

Brumfitt W, Ludlam H, Hamilton-Miller JMT, Gooding A. 1981. Lactobacilli do not cause frequency and dysuria a syndrome. Lancet. 2:393–395

Burkhardt O, Köhnlein T, Pletz M, Welte T. 2005. *Saccharomyces boulardii* induced sepsis: successful therapy with voriconazole after treatment failure with fluconazole. Scand J Inf Dis. 37:69–72.

Charteris WP, Kelly PM, Morelli L, Collins JK. 1998. Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. J Food Prot. 61:1636–1643.

Coeuret V, Gueguen M, Vernoux JP. 2004. Numbers and strains of lactobacilli in some probiotic products. Int J Food Microbiol. 97:147–156.

De Groote MA, Frank DN, Dowell E, Glode MP, Pace NR. 2005. *Lactobacillus rhamnosus* GG bacteremia associated with probiotic use in a child with short gut syndrome. Ped Infect Dis J. 24:278–280.

Dessart SR, Steenson LR. 1991. High frequency intergeneric and intrageneric conjugal transfer of drug resistance plasmids in *Leuconostoc mesenteroides* ssp *cremoris.* J Dairy Sci. 74:2912–2919.

Dickgieber U, Weiss N, Fritsche D. 1984. *Lactobacillus gasseri* as the cause of septic urinary infection. Infection. 12:14–16.

Doron S, Snydman DR. 2015. Risk and safety of probiotics. Clin Infect Dis. 60(Suppl 2):129–134.

European Food Safety Authority. 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA J. 10(2740):1–10.

Fukao M, Yajima N. 2012. Assessment of antibiotic resistance in probiotic lactobacilli. In: Pana M, editor. Antibiotic resistant bacteria – A continuous challenge in the new millennium. London (UK): IntechOpen [Internet]. doi: 10.5772/30903; [cited 2017 December 20]. Available from: http://www.intechopen.com/books/antibiotic-resistant-bacteria-a-continuous-challenge-in-the-new-millennium/assessment-of-antibiotic-resistance-in-probiotic-strains

Gevers D, Danielsen M, Huys G, Swings J. 2003a. Molecular characterization of *tet*(M) genes in *Lactobacillus* isolates from different types of fermented dry sausage. Appl Environ Microbiol. 69:1270–1275.

Gevers D, Huys G, Swings J. 2003b. *In vitro* conjugal transfer of tetracycline resistance from *Lactobacillus* isolates to other Gram-positive bacteria. FEMS Microbiol Lett. 225:125–130.

Global Market Insights [Internet]. 2017. Los Ageles (USA): GlobeNewswire; [cited 2018 March 14]. Available from: globenewswire.com/news-release/2017/10/10/1143574/0/en/Probiotics-Market-to-exceed-65bn-by-2024-Global-Market-Insights-Inc.html

Gueimonde M, Ouwehand AC, Salminen S. 2004. Safety of probiotics. Scand J Nutr. 48:42–48.

Guo H, Pan L, Li L, Lu J, Kwok L, Menghe B, Zhang H, Zhang W. 2017. Characterization of antibiotic resistance genes from lactobacillus isolated from traditional dairy products. J Food Sci. 82:724–730.

Harty DWS, Oakey HJ, Patrikakis M, Hume EBH, Knox KW. 1994. Pathogenic potential of lactobacilli. Int J Food Microbiol. 24:179–189.

Harty DWS, Patrikakis M, Knox KW. 1993. Identification of *Lactobacillus* strains from patients with infective endocarditis and comparison of their surface-associated properties with those of other strains of the same species. Microb Ecol Health Dis. 6:191–201.

Hempel S, Newberry S, Ruelaz A, Wang Z, Miles JN, Suttorp MJ, Johansen B, Shanman R, Slusser W, Fu N, et al. 2011. Safety of probiotics used to reduce risk and prevent or treat disease. Evid Rep Technol Assess. 200:1–645.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S. 2014. Expert consensus document "The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic". Nat Rev Gastroenterol Hepatol. 11:506–514.

Hoa NT, Baccigalupi L, Huxham A, Smertenko A, Van PH, Ammendola S, Ricca E, Cutting AS. 2000. Characterization of *Bacillus* species used for oral bacteriotherapy and bacterioprophylaxis of gastrointestinal disorders. Appl Environ Microbiol. 66: 5241–5247.

Horwitch CA, Furseth HA, Larson AM, Jones TL, Olliffe JF, Spach DH. 1995. Lactobacillemia in three patients with AIDS. Clin Infect Dis. 21:1460–1462.

Hove H, Mortensen PB. 1995. Influence of intestinal inflammation (IBD) and small and large-bowel length on fecal short-chain fatty-acids and lactate. Dig Dis Sci. 40:1372–1380.

Ishibashi N, Yamazaki S. 2001. Probiotics and safety. Am J Clin Nutr. 73(Suppl):465S–470S.

Johnson BR, Klaenhammer TR. 2014. Impact of genomics on the field of probiotic research: historical perspectives to modern paradigms. Antoine van Leeuwenhoek. 106:141–156.

Kalima P, Masterton RG, Roddie PH, Thomas AE. 1996. *Lactobacillus rhamnosus* infection in a child following bone marrow transplant. J Infect. 32:165–167.

Kochan P, Chmielarczyk A, Szymaniak L, Brykczynski M, Galant K, Zych A, Pakosz K, Giedrys-Kalemba S, Lenouvel E, Heczko PB. 2011. *Lactobacillus rhamnosus* administration causes sepsis in a cardiosurgical patient – is the time right to revise probiotic safety guidelines? Clin Microbiol Infect. 17:1589–1592.

Kunz AN, Noel JM, Fairchok MP. 2004. Two cases of *Lactobacillus* bacteremia during probiotc treatment of short gut syndrome. J Ped Gastroent Nutr. 38:457–458.

Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK. 2005. *Lactobacillus* sepsis associated with probiotic therapy. Pediatrics. 115:178–181.

Ledoux D, Labombardi VJ, Karter D. 2006. *Lactobacillus acidophilus* bacteremia after use of a probiotic in a patient with AIDS and Hodgkin`s disease. Int J STD AIDS. 17:280–282.

Lee TT, Morisset M, Astier C, Moneret-Vautrin DA, Cordebar V, Beaudouin E, Codreanu F, Bihain BE, Kanny G. 2007. Contamination of probiotic preparations with milk allergens can cause anaphylaxis in children with cow's milk allergy. J Allergy Clin Immunol. 119:746–747.

Lilly DM, Stillwell RH. 1965. Growth promoting factors produced by probiotics. Science. 147:747–748.

Liong MT. 2008. Safety of probiotics: translocation and infection. Nutr Rev. 66:192–202.

Liu Ch, Zhang Z, Dong K, Yuan J, Guo X. 2009. Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs. Biomed Env Sci. 22:401–412.

Łukasik J, Salminen S, Szajewska H. 2018. Rapid review shows that probiotics and fermented infant formulas do not cause D-lactic acidosis in healthy children. Acta Paediatr. 107:1322–1326.

Masco L, Huys G, De Brandt E, Temmerman R, Swings J. 2005. Culture-dependent and culture-independent qualitative analysis of probiotic products claimed to contain bifidobacteria. Int J Food Microbiol. 102:221–230.

Mathur S, Singh R. 2005. Antibiotic resistance in food lactic acid bacteria – a review. Int J Food Microbiol. 105:281–295.

McFarland LV. 2015. From yaks to yogurt: the history, development, and current use of probiotics. Clin Infect Dis. 60(S2):S85–S90.

Metchnikoff E. 1908. The prolongation of life. Optimistic studies. New York (USA): Putnam's Sons. p. 161–183.

Milazzo I, Speciale A, Musumeci R, Fazio D, Blandino G. 2006. Identification and antibiotic susceptibility of bacterial isolates from probiotic products available in Italy. New Microbiol. 29: 281–291.

Moneret-Vautrin DA, Morisset M, Cordebar V, Codreanu F, Kanny G. 2006. Probiotics may be unsafe in infants allergic to cow's milk. Allergy. 61:507–508.

Morelli L, Sarra PG, Bottazzi V. 1988. *In vivo* transfer of pAM beta 1 from *Lactobacillus reuteri* to *Enterococcus faecalis*. J Appl Bacteriol. 65:371–375.

Moubareck C, Gavini F, Vaugien L, ButelMJ, Doucet-Populaire F. 2005. Antimicrobial susceptibility of Bifidobacteria. J Antimicrob Chemother. 55:38–44.

Nawaz M, Wang J, Zhou A, Ma C, Wu X, Moore JE, Millar BC, Xu J. 2011. Characterization and transfer of antibiotic resistance in lactic acid bacteria from fermented food products. Curr Microbiol. 62:1081–1089.

Ooi LG, Liong MT. 2010. Cholesterol-lowering effects of probiotics and prebiotics: a review of *in vivo* and *in vitro* findings. Int J Mol Sci. 11:2499–2522.

Ouwehand AC, Saxelin M, Salminen S. 2004. Phenotypic differences between commercial *Lactobacillus rhamnosus* GG and *L. rhamnosus* strains recovered from blood. Clin Infect Dis. 39: 1858–1860.

Patel R, Cockerill FR, Porayko MK, Osmon DR, Ilstrump DM, Keating MR. 1994. Lactobacillemia in liver transplant patients. Clin Infect. 18:207–212.

Report FAO/WHO. 2001. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria [Internet]. p. 1–29; [cited 2017 November 15]. Available from: http://www.fao.org/tempref/docrep/fao/meeting/009/y6398e.pdf

Report FAO/WHO. 2002. Report of a Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food [Internet]. p. 36–48; [cited 2017 November 15]. Available from: http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf

Ruseler-van Embden JGH, van Lieshout LMC, Gosselink MJ, Marteau P. 1995. Inability of *Lactobacillus casei* strain GG, *L. acidophilus*, and *Bifidobacterium bifidum* to degrade intestinal mucus glycoproteins: clearing the way for mucosa-protective therapy. Scand J Gastroenterol. 30:675–680.

Saarela M, Mogensen G, Fonden R, Matto J, Mattila-Sandholm T. 2000. Probiotic bacteria: safety, functional and technological properties. J Biotechnol. 84:197–215.

Salminen MK, Rautelin H, Tynkkynen S, Poussa T, Saxelin M, Valtonen V, Järvinen A. 2004. *Lactobacillus* bacteremia, clinical signifiance, and patient outcome, with special focus on probiotic *L. rhamnosus* GG. Clin Infect Dis. 38:62–69.

Salminen MK, Tynkkynen S, Rautelin H, Saxelin M, Vaara M, Ruutu P, Sarna S, Valtonen V, Järvinen A. 2002. *Lactobacillus* bacteremia during a rapid increase in probiotic use f *Lactobacillus rhamnosus* GG in Finland. Clin Infect Dis. 35:1155–1160.

Salyers AA, Gupta A, Wang Y. 2004. Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol. 12:412–416.

Sanders ME, Akkermans LMA, Haller D, Hammerman C, Heimbach J, Hormannsperger G, Huys G, Levy DD, Lutgendorff F, Mack D, et al. 2010. Safety assessment of probiotic for human use. Gut Microbes. 1:164–185.

Sanders ME, Klaenhammer TR, Ouwehand AC, Pot B, Johanses E, Heimbach JT, Marco ML, Tenilä J, Ross RP, Franz C, et al. 2014. Effects of genetic, processing, or product formulation changes on efficacy and safety of probiotics. Ann N.Y. Acad Sci. 1309:1–18.

Schjørring S, Krogfelt KA. 2011. Assessment of bacterial antibiotic resistance transfer in the gut. Int J Microbiol [Internet]:1–10; [cited 2017 December 17]. Available from: http://dx.doi.org/10.1155/2011/312956

Schlegel L, Lemerle S, Geslin P. 1998. *Lactobacillus* species as opportunistic pathogens in immunocompromised patients. Eur J Clin Microbiol Infect. 17:887–888.

Senok AC, Ismaeel AY, Botta GA. 2005. Probiotics: facts and myths. Clin Microbiol Infect. 11:958–966.

Shao Y, Zhang W, Guo H, Pan L, Zhang H, Sun T. 2015. Comparative studies on antibiotic resistance in *Lactobacillus casei* and *Lactobacillus plantarum*. Food Control. 50:250–258.

Sharpe ME, Hill LR, Lapage SP. 1973. Pathogenic lactobacilli. J Med Microbiol. 6:281–286.

Sherid M, Samo S, Sulaiman S, Husein H, Sifuentes H, Sridhar S. 2016. Liver abscess and bacteremia caused by lactobacillus: role of probiotics? Case report and review of the literature. BMC Gastroenterol. 16:138.

Sherman ME, Albrecht M, DeGirolami PC, Kirkley SA, Wolf B, Eliopoulos GM, Rohrer RJ, Monaco AP. 1987. An unusual case of splenic abscess and sepsis in an immunocompromised host. Am J Clin Pathol. 88:659–662.

Snydman DR. 2008. The safety of probiotics. Clin Infect Dis. 46:S 104–111.

Sriskandan S, Lacey S, Fisher L. 1993. Isolation of vancomycin-resistant lactobacilli from three neutropenic patients with pneumonia. Eur J Clin Microbiol Infect Dis. 12:649–650.

Sybesma W, Molenar D, van Ijcken W, Venema K, Kort R. 2013. Genome instability in *Lactobacillus rhamnosus* GG. Appl Environ Microbiol. 79:2233–2239.

Tan KP, Yang M, Ito S. 2007. Activation of nuclear factor (erythroid-2 like) factor 2 by toxic bile acids provokes adaptive defense responses to enhance cell survival at the emergence of oxidative stress. Mol Pharmacol. 72:1380–1390.

Tan LT-H, Chan K-G, Lee L-H, Goh B-H. 2016. *Streptomyces* bacteria as potential probiotics in aquaculture. Front Microbiol. 7:79.

Temmerman R, Pot B, Huys G, Swings J. 2002. Identification and antibiotic susceptibility of bacterial isolates from probiotic products. Int J Food Microbiol. 81:1–10.

Teuber M, Meile L, Schwarz F. 1999. Acquired antibiotic resistance in lactic acid bacteria from food. Antoine Van Leeuwenhoek. 76:115–137.

Thompson C, McCarter YS, Krause PJ, Herson VC. 2001. *Lactobacillus acidophilus* sepsis in a neonate. J Perinatol. 21:258–260.

Tissier H. 1906. Treatment of intestinal infections using bacterial flora of the intestine. Crit Rev Soc Biol. 60:359–361.

Tsai Y-T, Cheng P-C, Pan T-M. 2012. The immunomodulatory effects of lactic acid bacteria for improving immune functions and benefits. Appl Microbiol Biotechnol. 96:853–862.

Tynkkynen S, Singh KV, Varmanen P. 1998. Vancomycin resistance factor of *Lactobacillus rhamnosus* GG in relation to enterococcal vancomycin resistance (*van*) genes. Int J Food Microbiol. 41:195–204.

Vahabnezhad E, Mochon AB, Wozniak LJ, Ziring DA. 2013. *Lactobacillus* bacteremia associated with pobiotic use in a pediatric patient with ulcerative colitis. J Clin Gastroenterol. 47: 437–439.

Van den Nieuwboer M, Brummer RJ, Guarner F, Cabana M, Morelli L, Claassen E. 2015. Safety of probiotics and synbiotics in children under 18 years of age. Benef Microbes. 6:615–630.

Van den Nieuwboer M, Claassen E, Morelli L, Guarner F, Brummer RJ. 2014. Probiotic and synbiotic safety in infants under two years of age. Benef Microbes. 5:45–60.

Van Leeuwen PAM, Boermeester MA, Houdijk APJ, Ferwerda ChC, Cuesta MA, Meyer S. 1994. Clinical significance of translocation. Gut. 35(Suppl):S28–S34.

Vankerckhoven V, Huys G, Vancanneyt M, Vael C, Klare I, Romond MB, Enteza JM, Moreillon P, Wind RD, Knol J, et al. 2008. Biosafety assessment of probiotics used for human consumption: recommendations from the EU-PROSAFE project. Trend Food Sci Tech. 19:102–114.

Venugopalan V, Shriner KA, Wong-Beringer A. 2010. Regulatory oversight and safety of probiotic use. Emerging Infect Dis. 11:1661–1665.

Vitetta L, Coulson S, Thomsen M, Nguyen T, Hall S. 2017. Probiotics, D-lactic acidosis, oxidative stress and strain specificity. Gut Microb. 8:311–322.

Zawistowska-Rojek A, Zaręba T, Mrówka A, Tyski S. 2016. Assessment of the microbiological status of probiotic products. Pol J Microb. 65:97–104.

Zheng M, Zhang R, Tian X, Zhou X, Pan X, Wong A. 2017. Assessing the risk of probiotic dietary supplements in the context of antibiotic resistance. Front Microbiol. 8:908.