PROBIOTICS AND THEIR USES IN CLINICAL PRACTICE – AN OVERVIEW

Mehmet İlktaç*[1], Hanin Tanjara[1], Sultan Öğmen[1], Gülden Çelik[1]

[1] Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus

Correspondence: mehmet.ilktac@emu.edu.tr, +903926302401

ABSTRACT

Probiotics**,** considered to have some benefits on human health when consumed in adequate amounts, are living microorganisms that can colonize intestine, mouth, mucous membranes, vagina, and skin. Probiotics have been tried for treating irritable bowel syndrome, enhancing immune system, maintaining balance in intestinal microbiota, preventing colorectal cancer, lactose intolerance, urinary tract infections, antibiotic-associated diarrhea and managing hepatic encephalopathy. They have been shown to display anti-hypercholesterolemic and antihypertensive impacts and have some positive effects on children and pregnant women. Their mechanism of action has been unexplored and related studies have become increasingly popular.

Lactobacillus and *Bifidobacterium* and the yeast *Saccharomyces cerevisiae* are microorganisms that are mostly utilized as probiotics. Lactic acid bacteria, including *Lactobacillus*species, can serve a dual function by being used in food fermentation and potentially imparting health benefits such as maintaining a healthy-immune system.

Safety of probiotics is a concern as they may result in bacteremia, fungemia, some side effects and may interact with immunosuppressive drugs leading to life threatening conditions. Further studies are needed to clarify the optimum dosage, duration of the treatment, usage of mix versus single-strain probiotics, cost effectiveness, hazards, counteractive effects to pathogens and usage in the treatment of various diseases.

Key words: Probiotic, *Saccharomyces cerevisiae*, *Lactobacillus*, *Bifidobacteria*

INTRODUCTION

Microorganisms colonizing certain parts of the body and living together with human without causing any disease under normal conditions, once named flora and now known as microbiome, interestingly outnumber the total number of human cells by ten-fold. Human microbiome, also called microbiota, consists of a mixture of genes and related products of trillions of bacteria, archae, microeucaryotes and viruses residing in different parts of the body and is considered as a barrier and a protective part of the human body. With recent developments in high-throughput DNA/RNA sequencing and computational technologies,

scientists have discovered uncultured microorganisms in the microbiome besides the cultured ones and stated that the diversity of human microbiome is much more than ever been thought. The “Human Microbiome Project”, which aims to characterize the components of microbiota in various parts of the human body by molecular methods so that effect of microbiome on human health and disease can be clarified, has led to many contributions to the science of human microbiome (Aagaard *et al*. 2015).

Probiotics are defined as “*live microorganisms conferring benefit to health when consumed in adequate amounts”* by Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) (Chen and Sears 2015).

Probiotics were first mentioned over a century ago by Elie Metchnikoff. Metchnikoff’s aim was to improve the health and dementia by controling the intestinal microbes using beneficial bacteria found in yogurt. In 1907, Metchnikoff anticipated that the acid producing bacteria in fermented milk products could prevent "fouling" in the colon and if consumed regularly could lead to a longer and healthier life (Mackowiak 2013). The first report on probiotics was published in 1965. Approximately 60% of more than 8000 probiotic related publications are human based studies. Various probiotics are currently marketed as food ingredients, dietary supplements, or “medical food” and international market of probiotics is enormous. However, the number of probiotics whose benefits have been proved by scientific studies is limited and vary from strain to strain and disease to disease (Aagaard *et al*. 2015).

Probiotics are live, beneficial microorganisms that are generally found in different anatomical locations in our body, e.g., intestine, mouth, viscous mucosal membranes, vagina and skin. *Lactobacillus* and *Bifidobacteria*, two frequently used probiotic bacteria, are found in the gastrointestinal (GI) microflora. In contrast to probiotics, prebiotics are indigestible substances (not living organisms) that may have beneficial effect on host by selectively stimulating the growth and/or activity of one or more but in general limited number of bacteria in the colon. The most commonly used prebiotics are inulin and fructooligosaccharide which are added to different foods containing fat and sugar. Addition of prebiotics to a formulation has been reported to stimulate the growth of only beneficial bacteria in the gastrointestinal tract. The treatment strategy in which probiotic and prebiotic are administered at the same time is called symbiotic (Chen and Sears 2015).

Probiotic preparations include highly variable microorganisms. Some probiotics include single microbes usually from the genera of *Lactobacillus, Streptococcus* and *Bifidobacterium* as bacteria and from the genus of *Saccharomyces* as fungi; whereas the others are composed of multiple distinct microbes. The commercial product VSL#3 that is composed

of eight strains of bacteria from the genera of *Bifidobacterium*, *Lactobacillus* and *Streptococcus* (*S. thermophilus*, *B. breve*, *B. longum*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, and *L. bulgaricus*) is one of the well-known example of such a probiotic containing a mixture of different bacteria. Apart from probiotics, lactobacilli and bifidobacilli are naturally present in fermented foodstuffs (e.g., yogurt, cheese, and sauerkraut), fermented vegetables, and olives. Probiotics is generally used as a supplement (pills, capsules, tablets and powders).Probiotics are becoming an attractive alternative treatment strategy as supplements due to their confirmed efficacy and safety in some diseases. However, the use of probiotics as supplements should be strictly regulated because of both some controversial results about the efficacy and safety and the lack of consensus on the optimum dosage (Chen and Sears 2015, Sanders 2009, Berman 2015).

Probiotics are named and classified by group, genus, species and strain as following:

Lactic acid bacteria (group)→*Lactobacillus* (genus)→*acidophilus* (species) →LA-5 (strain)

Bacteria and fungi that have received "Generally Recognized as Safe" (GRAS) status in United States are given in Table 1.

Table 1: Bacteria and fungi accepted as safe in probiotics by receiving GRAS ("Generally Recognized as Safe") Status in the United States (Chen and Sears 2015).

Bifidobacterium lactis strain Bb12 and *Streptococcus thermophilus* strain Th4
Bifidobacterium longum BB536
Bifidobacterium animalis subsp. *lactis* strains Bf-6, HN019, Bi-07, B1-04, and B420
Carnobacterium maltaromaticum strain CB1 (viable and heat treated)
Lactobacillus acidophilus, Lactobacillus lactis and *Pediococcus acidilactici*
Lactobacillus acidophilus NCFM
Lactobacillus casei subsp. *rhamnosus* strain GG
Lactobacillus casei strain Shirota
Lactobacillus reuteri strain DSM 17938
Lactobacillus reuteri strain NCIMB 30242
Lactobacillus rhamnosus strain HN001
Lactobacillus rhamnosus strain HN001 produced in a milk-based medium
Propionibacterium freudenreichii ET-3, heat killed
Saccharomyces cerevisiae strain ML01, carrying a gene encoding the malolactic enzyme from *Oenococcus oeni* and a gene encoding malate permease from *Schizosaccharomyces pombe*

Characteristics of Optimum Probiotics

The important characteristics of probiotic microorganisms were defined as follows by Food and Agricultural Organization of United Nations (FAO) and WHO (Chen and Sears 2015):

(1) Taxonomically categorized probiotics have to be placed in an internationally recognized culture collection

(2) Probiotics have to sustain their viability and stability after culturing, manipulation and during storage before ingestion

(3) Probiotics have to be resistant to gastric acid, biliary and pancreatic secretions

(4) A host response has to be induced once ingested

(5) They must have a functional or clinical benefit when consumed by the host in adequate amounts;

(6) They have to be safe in terms of both side effects and antibiotic resistance pattern.

Lactobacillus GG, one of the most frequently used probiotics, is chosen for probiotic intervention specifically because it survives the passage through the stomach, the acidic part of upper gastrointestinal system, multiplies in the intestine, adheres strictly to epithelial cells in vitro and prevents adherence of *E. coli* O157:H7 (enteropathogenic strain of *E. coli* that can lead to haemolytic uremic syndrome as a complication after foodborne infections), and produces some chemicals that are effective against some other pathogenic bacteria (Sanders *et al*. 2010).

Dosage

The effectiveness of probiotics depends on their doses. Because of the variety of probiotic organisms and variety in their administration route and delivery, no exact dosage can be provided for probiotics in general. Doses of probiotics are generally expressed in “colony forming units per millilitre” (CFU/ml). Although the effective dose varies among different probiotics, generally the minimum concentration of probiotic microorganism is accepted to be 10^6 cfu/ml. Studies related with the dose response effect of probiotics is also controversial depending on the type of the disease they are used for. Dose response effect has been determined for some diseases such as antibiotic associated diarrhea (AAD), whereas such an effect has not been determined for some other diseases (Sanders 2009, Ouwehand 2016, Kechagia *et al*. 2013). Since it changes from probiotic to probiotic, the concentration (dose) of the probiotic should be mentioned on the label. In addition to the dose of the probiotic, the label of probiotic preparations should include the genus, species and strain names of probiotics, the

end of the shelf-life of each probiotic strain, health claim, safety storage conditions and customer information (Chen and Sears 2015, Sanders 2009, Sanders *et al*. 2010).

Mechanisms of Action

Although the mechanisms of actions of probiotics have not fully been explored yet, clinical benefits of probiotics are thought to be due to enhancement of the epithelial barrier, inhibition of pathogen adhesion via adhering and occupying receptors on intestinal mucosa, secretion of antimicrobial substances and competitive (e.g., competition for nutrient) exclusion of pathogenic microorganisms. By colonizing the gastrointestinal system, probiotics interact not only with gastrointestinal cells but also with the elements of mucosal or systemic immune response. Probiotic bacteria are thought to interfere with natural killer cells, cytokine secretion, macrophage activation, and secretory IgA activity. The action of mechanisms of probiotics can be investigated under four main titles (Berman 2015, Reid 2016, Bermudez-Brito *et al*. 2012, Gogineni *et al*. 2013, Chen and Sears 2015):

a) Nutrient Competition: Within the gut, probiotics tend to compete with pathogenic microorganisms for the nutrients that they need in common for their growth and reproduction. Therefore, the more the beneficial microorganisms in the gut, the more the competition there will be between beneficial and pathogenic microorganisms.

b)Adhesion Competition: By adhering strongly to adhesion sites and occupying receptors on gut mucosa, probiotic bacteria prevent or limit the colonization (the first step of the infection) of bacteria that would lead to infections like acute gastroenteritis or deficiency in digestion and nutrient absorption within the gut.

c) Antimicrobial effect: Antimicrobial effect can either be achieved by bacteriocins, peptides or proteins produced by many species of lactic acid bacteria that have antimicrobial activity against other bacteria, or by the production of organic acids which have direct antimicrobial activity or may show indirect antimicrobial effect by reducing the pH of the gut.

d) Enhancement in digestion: Probiotic microorganisms act like an element of microflora in the gut after they adhere and produce enzymes that aid in the breakdown of polysaccharides eventually resulting in the generation of energy. Moreover, these microflora organisms both ferment carbohydrates which have not been digested in the upper parts of the gut and produces vitamins supplying a secondary source to the host.

e) Immune modulation: Some probiotic organisms such as *L. casei* have been shown to increase the secretion of immunoglobulin A; the main immunoglobulin that is responsible for the mucosal immunity, in the gut resulting in the reduced morbidity of bacterial gut

pathogens. *L. casei* has also shown to be related with reduction in the secretion of pro-inflammatory cytokines, thus showing an anti-inflammatory effect.

Side effects

In general, probiotics are accepted to be safe in case they are consumed in the right dose. However, theoretically, exposing humans to live microorganisms brings some risks. The potential side effects of probiotics can be summarized as follows (Berman 2015, Vandenplas *et al*. 2015, Doron and Snydman 2015):

a) Systemic infection: Lactobacillemia, the blood stream infection caused by *Lactobacillus*, has been reported as one of the most serious side effects of probiotics containing *Lactobacillus*. Probiotics have also been related with septicemia, fungemia and endocarditis. Immunocompromised people are the most important risk group for these probiotic related systemic infections.

b) Deleterious metabolic activities: The most dangerous deleterious metabolic effect related with the probiotics is related with D-lactate, produced by some probiotic strains, that interferes with the bile salt and lead to short bowel syndrome.

c) Over-stimulation of immune system: Although not confirmed by clinical studies, probiotics are suspected to have the potential to lead to inflammatory or autoimmune reactions as a result of their capability to stimulate immune system and induce cytokine secretion from various cells.

d) Gene transfer: Lactic acid bacteria have small extrachromosomal DNAs, called plasmids, which contain genes that result in the development of resistance to various antibiotics including tetracycline, chloramphenicol, macrolides, lincosamides and streptogramine. Plasmids are mobile DNA elements that can be transferred among different strains of bacteria by a type of recombination called conjugation. Conjugation results in the dissemination of antibiotic resistance among bacteria. Although such scientific evidence has not yet been found, possible resistance gene transfer via plasmids can lead to the dissemination of antibiotics resistance among the normal flora bacteria of the gut.

Clinical Applications of Probiotics

There are numerous studies related with the use of probiotics as one of the elements of the treatment in various diseases or clinical manifestations. Although probiotics have many indications, the most common indication for probiotics is acute diarrhea related with bacterial or viral infectious agents. Cochrane, a global network consisting of researchers, professionals and people interested in health and providing high quality systematic reviews, has numerous

valuable systematic data about the use of probiotics especially in gastrointestinal manifestations or infections (infectious diarrhea, AAD, *C. difficile* colitis, inflammatory bowel disease, necrotizing enterocolitis in preterm infants, irritable bowel syndrome (IBS) and collagenous colitis). Cochrane reviews also contain studies related with the use of probiotics in pediatric diarrhea, ulcerative colitis, Crohn's disease, hepatic encephalopathy, non-alcoholic fatty liver disease/steatohepatitis, hepatic postsurgical complications, allergic diseases, food hypersensitivity, eczema and bacterial vaginosis (Chen and Sears 2015, Berman 2015).

Some important examples of the clinical use of probiotics can be summarized as follows:

1. Diarrhea

a.) AAD: AAD and pseudomembranous enterocolitis are results of the overgrowth of toxigenic *Clostridium difficile* (recently it has been shown that overgrowth of *Klebsiella oxytoca* can also lead to AAD) after antibiotic (all types of antibiotics but especially clindamycin and beta-lactam antibiotics) consumption. AAD is among the most common side effects of antibiotic therapy in hospitalized patients; especially those hospitalized in intensive care units. Probiotics are frequently used as a part of the treatment in patients with AAD. The microbial ingredient of the probiotic used may change according to the type of the antibiotic that has led to AAD. For example, in clindamycin related AAD *B. longum* + *Lactobacillus* preparations; whereasin *β-lactam or tetracycline*-associated diarrhea *S. boulardii* preparations can be preferred (Berman 2015, Issa and Moucari 2014).

b.) Traveler's diarrhea

Traveler's diarrhea (TD), one of the most frequently detected forms of diarrhea, is detected among international travellers either during the travel or just after the return. It is an acute diarrhea that is clinically presented as watery stool more than three times a day together with one or more enteric symptoms, e.g., abdominal cramps. In some studies, probiotics have shown to be effective in the treatment and prevention of TD. In addition, some probiotics have been shown to reduce the risk of severe necrotizing enterocolitis in preterm infants and shorten the duration of infectious diarrhea. However, the use of probiotics in TD cases still remains controversial in general (Teitelbaum 2005, Chen and Sears 2015, McFarland 2007).

2. IBS

IBS is a disorder of chronic abdominal pain, changed bowel habit and abdominal inflation. There is now increasing evidence relating IBS to alterations in GI microbiota (Parkes *et al*. 2010) or to the interaction among the intestinal bacteria, gut barrier and the intestinal

immune system. Probiotics were suggested to repair the imbalance in the micro-flora and improve the quality of life in IBS patients (Thompson 2016). Different therapeutic approaches including probiotics have been used for the treatment of IBS. However, only a few of probiotics have been successful or had no side effect. Not all probiotics have been reported to be beneficial for IBS. Therefore, to select the specific strain whose efficacy has been shown was reported to be an important factor in the treatment (Moraes-Filho and Quigley 2015). On the other hand, the use of probiotic containing a mixture of different strains rather than single strain probiotics can be more effective in IBS. Together with data supporting the infectious diseases as a possible aetiology of IBS, multiple factors are now being thought to play a role in its development. The difference in the efficacy of different probiotics may be as a result of the multi-nature characteristic of the disease. Further studies are required in order to determine the most effective species/strains, the optimal dose and to determine whether a probiotic including a combination of different strains is better than a single strain (Thompson 2016, Moraes-Filho and Quigley 2015, Meier 2010).

3. Obesity

Obesity is a serious public health concern and is the most frequent cause of other health problems in developed countries. Obesity that can lead to the development of metabolic diseases both in adults and children is related with some external and internal factors including dietary habits, lifestyle and genetics. Human intestine has trillions of microbes that make up the gut microflora which plays an important role in human metabolism. Any change in the balance of the components of microflora may lead to obesity and metabolic syndrome (John and Mullin 2016). Diet has been shown to have a marked effect on the gut environment including gut transit time and pH. Therefore, a change in the intake of macronutrients including carbohydrates, proteins and fats can affect the composition of the intestinal microbiota (Maukonen and Saarela 2015). Probiotics have been suggested to play a role in the treatment of obesity and related metabolic disorders like hyperglycemia and dyslipidemia (Sanchez *et al.* 2015). In many studies carried out on animals and a few studies on humans, the genera *Lactobacillus* and *Bifidobacterium* have been reported to have multiple beneficial effects on metabolic syndromes, such as reduction in weight and visceral fat, and improvement in glucose tolerance (Park and Bae 2015, Teixeria *et al*. 2013).

Results of the studies related with the effect of probiotics on weight management are controversial. Besides studies reporting the weight reduction upon the use of probiotics, there are some studies in which probiotics have been reported not to have beneficial effect on the weight over the long term. On the other hand, in some studies chemicals (prebiotics) that induce

the growth and/or activity of commensal microorganisms have been reported to have more beneficial effect than probiotics because the latter may not lead to a change in gut microbiota in humans. Further studies should be conducted to clarify the effects of probiotics and gut microbiota on body weight and obesity (Sanchez *et al*. 2015, Park and Bae 2015).

4. Oral diseases

Oral cavity is an excellent niche for the colonization of bacteria due to the presence of suitable factors (e.g., nutrient, pH, and humidity) that are required for their growth. Bacterial colonization of the oral cavity in turn may result in dental caries, periodontal disease and halitosis.

Probiotics have the ability to modify the micro-environment by changing the environmental factors, such as the pH and the oxidation–reduction potential that are crucial for the colonization of bacteria. Moreover, bacteria used as probiotics both secrete several antimicrobial substances including organic acids, hydrogen peroxide, bacteriocins and compete with pathogenic microorganisms for the receptors that are present on human cells as bacterial adhesion sites (Bonifait *et al*. 2009). Probiotics act against caries via following mechanisms (Meurman 2005):

(a) Prevention of the adhesion, the first phase of infection, of bacteria to tooth surfaces via forming biofilm on tooth surface

(b) Competing with caries-related bacteria in terms of nutrient and attachment site

(c) Secreting antimicrobial substances so that they lead to reduction in bacterial colonization.

(d) Enhancing local and systemic immune system

(e) Regulating mucosal permeability

Probiotics obtained from dairy products such as *S. thermophilus* and *L. lactis* are capable to integrate themselves into a biofilm already formed on the hydroxyapatite surface. Such an integration may be followed by the interference with the developmental stages of caries by *Streptococcus sobrinus* (Singh *et al*. 2013). Probiotics utilized for dental caries selectively remove only the harmful pathogen without affecting the rest of the oral microenvironment (Hauikoja 2010).

Periodontal diseases are among the most common diseases encountered in dentistry. Gingivitis and periodontitis are two types of periodontal diseases. Gingivitis is the local inflammation of the gingiva whereas periodontitis is progressively related with the all supporting tissues of teeth including the alveolar bone. Periodontitis may be a result of various putative pathogens, including *Aggregatibacter*, *Tannerellaforsythia* and

Porphyromonasgingivalis (Gupta 2011). Probiotics residing in the gingival sulcus have been reported to have some beneficial effects such as reducing the number of the pathogen and strengthening the epithelial barrier, thus contributing to decreased susceptibility to infection (Singh *et al*. 2013).

Anaerobic microorganisms in the oral microflora are one of the leading causes of halitosis. These bacteria convert salivary and food proteins into amino acids which are in turn transformed into volatile sulphur compounds like hydrogen sulphide and methanethiol that are responsible for unpleasant odour. *Streptococcus salivarius* is used as the part of gums or lozenges to produce bacteriocins that would decrease the number of anaerobic bacteria that produce volatile sulphur compounds (Bonifait *et al*. 2009). In addition, lactic acid bacteria can also alter the oral microflora via the antimicrobial by-products such as carbon peroxide, organic acids, diacetyl, hydrogen peroxide, low molecular weight antimicrobial substances, bacteriocins and adhesion inhibitors that they generate (Meurman 2005).

Probiotic therapy is an interesting field and has recently gained attraction in oral medicine and dentistry. However, further researches especially related with their efficacy, action of mechanisms in oral cavity, capability of colonizing oral biofilms and interference with the components of the biofilm are required (Devine and Marsh 2009).

5. Skin diseases

Skin, surrounding the outer surface of the body, has a large surface area and is always exposed to physical, chemical, bacterial and fungal damages. Intact skin is a good barrier for microorganisms preventing their entrance into the body. In certain diseases such as eczema and acne, the barrier function of the skin is disrupted increasing the susceptibility of the affected person to infections. Eczema, a skin disease with itchy red rash, is more common in childhood than in adulthood. New treatment strategies depending on the alteration of the gut flora or reduction of inflammation in the gut have been implemented to control eczema, reduce its effect on the quality of life, decrease financial costs to the community and reduce its symptoms (Roudsari *et al*. 2013).

Preparations consisting of *Lactobacillus* spp. have attracted a considerable interest recently because people with eczema have been detected to show differences in terms of the components of their intestinal microbiota with respect to those without eczema. Although not proved yet, probiotics consisting of *Lactobacillus* GG have been shown to reduce the risk of eczema by two fold (Wickens *et al*. 2008). The mechanism of action of probiotics in treating eczema is thought to be related with reduction in the intestinal inflammation and intestinal

permeability leading to a change in antigen presentation in the gut-associated lymphoid tissue (Boyle *et al*. 2009).

The debate about the efficacy of probiotics on skin diseases is still going on. Several scientists who carried out studies on eczema patients using different probiotic strains reported that probiotics did not have any beneficial effect on whereas other scientists reported that probiotics could have positive effect on the treatments of skin diseases like atopic dermatitis depending on multiple factors, like the dosage, the time of administration of specific probiotic strains and the duration of exposure (Boyle *et al*. 2009, Boyle *et al*. 2008, Rather *et al*. 2016). To sum up, updated information lacks strong evidence to support the use of probiotics in the treatment of skin diseases.

6. Liver diseases

Liver can directly be influenced when the gut microbiota is altered due to the entry of gut bacteria or their metabolites into the liver through the liver-gut axis and the portal vein. The gut flora has been shown to be different in terms of its composition in patients with non-alcoholic fatty liver disease (NAFLD) compared to healthy human. Gut microbiota has been suggested by several scientists to be responsible for the progression of NAFLD to non-alcoholic steatohepatitis. Patients who had advanced liver cirrhosis can develop hepatic encephalopathy (HE) as a complication and HE is thought to be related with toxic metabolites (especially ammonia) produced by the gut flora (Minemura and Shimizu 2015).

Because of their ability to alter the gut flora and/or permeability of the gut, thus leading to a decrease in the production and absorption of ammonia, probiotics have been reported to be used in the treatment of HE (Minemura and Shimizu 2015).

Via endotoxemia and increasing the oxidative stress in the gut, alcohol abuse induces liver injury leading to liver fibrosis, fatty liver, alcoholic hepatitis and liver cirrhosis. Because these clinical manifestations are followed by a change in the gut microbiota, probiotics have been suggested as promising supplements as the part of the treatment of such liver diseases. After the administration of probiotics that alter the gut microbiota, lowered levels aspartate transaminase (AST) and alanine transaminase (ALT) in the blood of patients with liver diseases related with chronic alcohol consumption have been reported in numerous studies. Preparations including *L. plantarum* which decrease the inflammation in the intestine caused by increased oxidative stress are among the probiotics that have a potential therapeutic effect. On the other hand, bacteria-free culture supernatant of *L. rhamnosus* GG was also confirmed to suppress the alcohol-induced intestinal permeability, endotoxemia and liver injury. Thus, still it remains

uncertain whether live strains are compulsory or only the products of these bacteria are sufficient for the treatment these diseases (Sharma and Singh 2016, Sharma *et al*. 2013).

7. Hypercholesterolemia

The ability of probiotics to interact with bile acid via different mechanisms has led to the development of new strategies in the treatment of increased cholesterol levels (hypercholesterolemia) in the body. Deconjugation of bile acids by bile salt hydrolase (BSH) is the main mechanism of probiotic bacteria for decreasing the level of cholesterol. However, such a treatment relying on the BSH enzyme produced by probiotics has side effects on the human body. Further approaches for reducing the side effects of probiotics in this field are required in order for probiotics to be used in the treatment of hypercholesterolemia (Pavlović *et al*. 2012).

8. Cardiovascular diseases

New treatment strategies for cardiovascular diseases have currently been investigated since these diseases are one of the main causes of premature mortality worldwide. These new strategies include the use of probiotics for decreasing triglyceride and high-sensitivity C-reactive protein (hs-CRP), an indicator of atherothrombotic and cardiovascular disease risk, and enhancing high density lipoprotein (HDL) without any alteration in total cholesterol or low density lipoprotein (LDL) cholesterol levels. Beneficial effects of probiotics and the role of microbiome in cardiovascular diseases have not yet been clarified definitely. Therefore, more studies are needed to understand the relationship between probiotics and cardiovascular diseases so that new strategies for the prevention and the management of heart diseases would be available (Sanaie *et al*. 2013, Ettinger *et al*. 2014).

9. Peptic ulcer

Peptic ulcer disease whose prevalence is high in many parts of the world develops as a result of the inflammation of gastric or duodenal mucosa generally due to factors such as prolonged consumption of NSAIDs, *H. pylori* infection, smoking and alcohol intake. All of these factors decreases or inhibits the secretion of mucus and bicarbonate leading to increased acid secretion that results in mucosal damage. Probiotics including *L. acidophilus* and *S. boulardii* have been reported to accelerate the healing of ulcers and those including *Lactobacillus johnsonii* La1 have been used in the treatment of *H. pylori* infections (Vomero and Colpo 2014, Khoder *et al*. 2016).

10. Nervous system diseases

Central nervous system (CNS) and gut have an integrative physiology that includes gut-brain-axis and nutrient, endocrine and immunological signals between the CNS and GI

tract. CNS is influenced by microbial components of the microbiota. Regulation of satiety via CNS-gut-microbiome signalling is the best example of such an interference. The signalling, which is a result of satiation-signalling peptides, changes according the type of the food consumed since the diet affects the components of gut microbiota. In turn, metabolical by-products of gut microbiota affect the secretion of these signalling peptides. Peptides secreted by enteroendocrine cells in response to by-products of microbiome are transported through the blood to the brain finally affecting the satiety centre (Wang and Kasper 2014).

Another example of the relationship between the gut microbiome and the CNS is related with psychological disorders such as anxiety and depression. Probiotics, especially *Bifidobacterium* and *Lactobacillus* spp. or a mixture of *L. rhamnosus* and *L. helveticus* strains, have been reported to have anti-depressant activity. The action of mechanism of probiotics in depression may be due to attenuation of pro-inflammatory cytokines, increase in the tryptophan level and production of neuro-active substances (Zhang and Abdullah 2013). Dementia, Alzheimer's disease being the most common type, is a type of nervous system disease that affects the person's daily functioning. Relation of microbiota with dementia via various mechanisms (e.g., leading to cardiovascular disease which has been shown to be a risk factor for dementia or leading to the development of some metabolical abnormalities like high blood sugar and increase in the body fat) and the potential use of probiotics, especially those that lead to the production of B vitamins, in the treatment of dementia have recently been reported (Rashad *et al*. 2016).

11. Urinary tract infections (UTIs)

UTIs, one of the most commonly detected bacterial infections that may be responsible for renal failure in long term, are among the most important causes of health expenditure due to the need for the antibiotic treatment. In addition, the use of antibiotic in the treatment of UTIs is the main reason of the development of antibiotic resistance in bacteria, a worldwide public health concern that has been gradually increasing since the last decade. An alternative treatment method that would replace antibiotherapy would definitely lead to a decrease in the antibiotic resistance rate because it has been proved that the less the antibiotic consumption is, the lower the antibiotic resistance rate would be. Probiotics are therefore supplements which have gained interest as an alternative treatment for UTIs.

S. boulardii was reported to be possibly effective in the prevention of UTIs caused by *E. coli* indirectly, via leading to reduction in the number of *E. coli* colonizing the colon, since the source of most of *E. coli* cystitis is stool (Akil *et al*. 2006). In addition, probiotics consisting of

Lactobacillus spp. have been reported to be an effective prophylactic agent for the prevention of recurrent pyelonephritis in infants by preventing the adhesion of uropathogenic bacteria, changing the pH of the urinary tract towards acidic, leading to the accumulation of hydrogen peroxide that has antimicrobial activity in the urinary tract and regulating mucosal immunity (Lee *et al*. 2016).

In addition, when used together in combination with antibiotics, probiotics may prevent the side effects of antibiotics by decreasing the risk of super-infections of gut or vagina by *C. difficile* or *Candida albicans*, respectively (Reid 2006).

12. Cancer

Cancer, the result of abnormal proliferation of cells in the body, is one of the most common causes of human death worldwide and is a result of the combination of genetic and environmental factors such as physical, chemical, and biological carcinogens, dietary factors and lifestyle (Saber *et al*. 2016).

Several studies have reported that different dietary habits and lifestyles may be related with cancer, especially with colorectal cancer (CRC), because these factors can disrupt the balance of the components of intestinal microflora (Ucello *et al*. 2012, Raman *et al*. 2013). Moreover, in some of studies investigating the efficiency of probiotics in cancer prevention, it was reported that probiotics can be effective in the prevention of cancer via mechanisms including the induction of the host's immune system, competition with putrefactive and pathogenic microbiota that can be related with the development of cancer, regulation of apoptosis and cell differentiation via anti-proliferative effects, inhibition of tyrosine kinase signalling pathways and fermentation of undigested foods (Motevaseli *et al*. 2017, Ucello *et al*. 2012, Raman *et al*. 2013, de Moreno de LeBlanc et al. 2007). In addition to their ability of preventing cancer development, it has been reported in a systematic review that the use of probiotics (*Lactobacillus* or *Bifidobacterium* spp.) in cancer patients is also effective for reducing the frequency of diarrhea, the most frequently encountered side effect of chemotherapy or radiotherapy (Redman *et al*. 2014).

Some bacteria that reside in intestine can convert some pro-carcinogens into carcinogens that would lead to cancer. Probiotics may inhibit the growth of these harmful bacteria so that they lead to the reduction in the concentration of carcinogens. Such an action of mechanism of probiotics, especially lactic acid bacteria, has been reported in colon cancer and breast cancer cases (de Moreno de LeBlanc et al. 2007) .

Probiotics containing yeasts such as *S. cerevisiae*, *S. boulardii* and *Candida spp.* have been reported to be more resistant in terms of penetration of the upper parts of GI tract and to

be more effective in the stimulation of immune system when compared to bacteria containing probiotics. Therefore, they may protect the host against pathogenic bacteria and their toxic compounds much more efficiently than bacteria containing probiotics. In addition, some fungal products, such as β-glucan, have shown to have possible beneficial effects on cancer development via the induction of immune system. Whereas studies investigating the anti-cancer effect of bacteria containing probiotics are numerous, those related with anti-cancer effects of yeast containing probiotics are limited. Therefore, further evaluations of yeast containing probiotic related with the prevention of different types of cancer are required (Saber *et al*. 2016).

In addition to their possible beneficial effects on cancer development, probiotics have also been reported to have some possible side effects, some of which are serious, on cancer patients. Currently, neutropenic cancer patients in United Kingdom are suggested not to use probiotic preparations. *Saccharomyces* related fungemia and *L. acidophilus* related bacteremia have been reported in cancer patients using related probiotics. In addition to blood stream infections, probiotics have also been reported to be responsible for the progression of malignancy (Redman *et al*. 2014).

13. Children diseases

A lot of studies have estimated the role of probiotics in critically ill children especially for the treatment and prevention of necrotizing enterocolitis (NEC), otitis media (OM), AAD, ventilator-associated pneumonia (VAP), invasive candidiasis and candida colonization (Singhi and Kumar 2016, Niittynen *et al*. 2012).

Probiotics may be alternative choice in the prophylaxis and the management of infectious diseases. In some studies, probiotic nasal sprays containing *Streptococcus sanguis*, *S. oralis* and *S. mitis* has been reported as an alternative treatment for otitis media, a bacterial infection which is common in children, whereas no effect has been detected in other studies. Similar to adults, the administration of a mixture of probiotics for one week could prevent and treat AAD in children. Although probiotics have been shown to be effective in the treatment of some childhood diseases, more studies are needed to clarify the duration of treatment, cost effectiveness and effectiveness of a mix versus single-strain probiotics. In addition, the safety of probiotics (the risk of bacteremia, fungemia, and sepsis) in critically ill, especially immunocompromised children has not been evaluated yet (Singhi and Kumar 2016, Niittynen *et al*. 2012).

14. Pregnant and lactating women

Pregnancy is a period that contains many physiological and immunological changes in order to provide a suitable environment for the fetus. Physiological changes in the gut and vagina during pregnancy lead to changes in the microbiota in these locations affecting the health of pregnant women. Orally or vaginally administered probiotics have been found to be effective on women during early or late periods of pregnancy, and during the lactation period (Gomez Arango *et al*. 2015).

Recently, several studies have confirmed that probiotic supplements taken during the pregnancy enhance mucosal immunity, regulate vaginal and gut microflora, improve metabolic activity and decrease urogenital infections in women. Probiotics (especially those containing *Lactobacillus*) were found to be effective in pregnant women with constipation in the early stages of pregnancy without any increase in the adverse pregnancy outcomes (Lee *et al*. 2012). The data about the efficacy of probiotics in the late stages, especially the last trimester, of pregnancy is limited. Pregnant women in their last trimester using the probiotic VSL #3 (a mixture of *Lactobacillus*, *Bifidobacterium* and *Streptococcus* strains) have reported to have higher concentration of anti-inflammatory cytokines IL-4 and IL-10 when compared to those who do not use (Vitali *et al*. 2012). Probiotics can also be effective for pregnant women with diabetes and obesity via balancing the microbiota composition.

Bacterial vaginosis (BV), common inflammation of the vagina, is as a result of the reduction in the number of *Lactobacillus* spp. found in the normal flora of vagina. Probiotics, especially those including *Lactobacillus* spp., have been used to normalize the microbiota of vagina so that BV is eradicated. Moreover, the use of probiotics together with metronidazole in bacterial vaginosis has also been investigated. Patients who used vaginal or oral probiotics together with metronidazole were reported to be at lower risk for the recurrence of BV than those who used only metronidazole (Homayouni *et al*. 2014, Bodean *et al*. 2013, Onderdonk *et al*. 2016). However, further studies are required in order to clarify the efficacy of probiotics in these diseases.

In neonate perspective, probiotics were reported to be rarely absorbed systematically and couldn't reach into the breast milk leading to any adverse effect on infant (Elias *et al*. 2011). Moreover, probiotic use during pregnancy was reported to lead to a change in the cytokine composition of the breast milk and induce the secretory IgA production in infant resulting in the improvement of the gastrointestinal function of the infant (Baldassarre *et al*. 2016).

15. Other Applications

Apart from applications in human, probiotics have applications in veterinary medicine and aquaculture as well. Probiotics have been used as a dietary supplement in animals to prevent and control pathogenic bacterial colonization. The challenge facing scientists in this field is the presence of various different species of bacteria in the gut of different animals reflecting the diversity of the composition of fecal microbiota among animals. A mixture of probiotic strains is used to cover more types of microbial pathogens. The mixture has to have specific characteristics such as possessing antimicrobial activity, surviving in the gastrointestinal tract and adhesion (Gaggia *et al*. 2010). *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Lactococcus*, *Streptococcus* and *Saccharomyces* species have been studied as probiotics in animals. Probiotics have also been used as a tool to control diseases in aquaculture that is the cultivation of aquatic organisms in controlled water environment. More studies are needed to ensure the role of probiotics in both fields (Edun and Akinrotimi 2011).

DISCUSSION

Probiotics which have attracted high interest of consumers recently are among the most popular products on the nutraceutical market or pharmacies and are considered as a type of functional food that are easy to reach and have little side effects when used correctly (38). Evidence was present about the benefits of probiotics when used at the right dose, duration and when suitable strains and species are chosen for specific diseases. However, rigorous clinical trials to ascertain the health benefits of most of the commercially available preparations on many clinical manifestations have not been done yet. Most probiotic preparations are currently marketed as food ingredients, dietary supplements, or "medical food." Yogurt products labelled as containing "live and active cultures" are supplemented with one or more probiotics (Chen and Sears 2015, Berman 2015).

WHO and FAO define probiotics as live microorganisms that enhance the health of the host when consumed in adequate amounts (Chen and Sears 2015). The definition brings out the priority of scientifically supported data related with health benefits of these products.Therefore, health benefits of probiotics have to be documented in well-designed, controlled clinical trials. However, only a few of marketed probiotics meet this standard. Moreover, the safety issue of most of the probiotics is still unclear due to the lack of enough number of studies about the possible negative effects of probiotics on the host. Further researches related with probiotic development, use and safety are warranted (Chen and Sears 2015, Berman 2015, Sanders *et al*. 2010).

Nowadays, probiotics are widely consumed in many countries and clinical studies related with their efficacy in the treatment and prevention of various diseases have still been going on. However, further in-vitro and clinical studies are required for clarifying the their mechanisms of action, benefits and side effects (Boyle *et al*. 2006).For the future, there is a need for obtaining probiotic foods from non-dairy environments like almond milk fermentation. New non-dairy probiotic candidates like fruits and vegetables are being investigated (Bernat *et al*. 2015).

Gene technology and genomic studies will play a role in rapid search and the development of new strains which might increase insight into the mechanisms and the functionality of probiotics that would offer promise for development of novel therapeutics (Syndman 2008). Advancements in the immunological or physiological properties of probiotics can be succeeded by genetic modification leading them to be used as vaccine vectors or mucosal delivery systems. Such a use of genetically modified strains is still limited because of lack of safety related studies (Syndman 2008).

CONCLUSION

Even though there is a substantial increase in the number of studies related with probiotics, the need for high number of volunteers for in-vivo studies to be conducted, the high number of different species of microbes to be covered, cost and ethical considerations slow down the clinical trials. In general, health organizations accept probiotics as “food supplements”; *not as drugs*. Therefore, at least for now, probiotics should not be used to replace the antimicrobials for the treatment of any infection and the un-controlled use of probiotics should be avoided, especially for the diseases in which their efficacy has not been proved yet.

Although the preliminary studies are promising, because of the lack of health benefits and safety issues for most of the preparations, clinicians and pharmacists should be aware of the quality-control issues in probiotic manufacturing, the limitations of probiotic use in some patients (immunocompromised patients, individuals at extreme ages and for those with central venous catheters, disrupted mucosal barriers, short bowel syndrome, abnormal cardiac valves, prosthetic joints, valves or prosthetic materials) and potential adverse consequences of probiotics and explain such important topics to their patients. Further clinical trials related with probiotics will add to the field of new treatment strategies for various diseases.

REFERENCES

Aagaard K, Luna K R, Versalovic J (2015). The human microbiome of local body sites and their unique biology. In Bennett JE, Dolin R, Blaser MJ and Parta M. (eds). *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases,* 8.th ed.,p.11-17 Saunders, Elsevier USA.

Akil I, Yilmaz O, Kurutepe S, Degerli K, Kavukcu S (2006). Influence of oral intake of *Saccharomycesboulardii* on *Escherichia coli* in enteric flora. *Pediatr Nephrol***21**(6):807-810.

Baldassarre ME, Di Mauro A, Mastromarino P, Fanelli M (2016). Administration of a multi-strain probiotic product to women in the perinatal period differentially affects the breast milk cytokine profile and may have beneficial effects on neonatal gastrointestinal functional symptoms. A randomized clinical trial.*Nutrients* **8**(11).pii: E677.

Berman J (2015). Complementary and Alternative Medicines for Infectious Diseases.In Bennett JE, Dolin R, Blaser MJ and Parta M. (eds). *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 8.th ed.., p.598-604, Saunders, Elsevier USA.

Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A (2012).Probiotic mechanisms of action.*Ann Nutr Metab* **61**:160-174

Bernat N, Chafer M, Chiralt A, Gonzalez-Martinez C (2015). Development of a non-dairy probiotic fermented product based on almond milk and inulin. *Food Sci Technol Int* **21**(6):440-453.

Bodean O, Munteanu O, Cirstoiu C, Secara D, Cirstoiu M *(*2013). Probiotics--a helpful additional therapy for bacterial vaginosis. *J Med Life* **6**(4):434-436.

Bonifait L, Chandad F, Grenier D (2009). Probiotics for oral health: myth or reality?. *J Can Dent Assoc* **75**(8):585-590.

Boyle RJ, Robins-Browne RM, Tang ML (2006). Probiotic use in clinical practice: what are the risks? *Am J Clin Nutr* **83**(6):1256-1264.

Boyle RJ, Bath-Hextall FJ, Leonardi-Bee J, Murrell DF, Tang ML (2009). Probiotics for the treatment of eczema: A systematic review. *Clin Exp Allergy***39**(8):1117-1127.

Boyle RJ, Bath-Hextall FJ, Leonardi-Bee J, Murrell DF, Tank MLK(2008). Probiotics for treating eczema. *Cochrane Database Syst Rev* doi: 10.1002/14651858.CD006135.pub2.

Chen LA, Sears CL (2015). Prebiotics, probiotics, and synbiotics. In Bennett JE, Dolin R, Blaser MJ and Parta M. (eds). *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases,* 8.th ed.,p.19-25, Saunders, Elsevier USA.

de Moreno de LeBlanc A, Matar C, Perdigón G (2007). The application of probiotics in cancer. *Br J Nutr* **98**(Suppl 1):S105-S110.

Devine DA, Marsh PD (2009). Prospects for the development of probiotics and prebiotics for oral applications. *J Oral Microbiol* **1**:10.

Doron S, Snydman DR (2015). Risk and safety of probiotics. *Clin Infect Dis* **60**(Suppl 2)*:*S129-S134.

Edun OM, Akinrotimi O (2011). The use of probiotics in aquaculture. *Nig J Biotech***22**:34-39.

Elias J, Bozzo P, Einarson A (2011). Are probiotics safe for use during pregnancy and lactation?. *Can Fam Physician***57**(3):299-301.

Ettinger G, MacDonald K, Reid G, Burton JP (2014). The influence of the human microbiome and probiotics on cardiovascular health. *Gut Microbes* **5**(6):719-28.

Gaggìa F, Mattarelli P, Biavati B (2010). Probiotics and prebiotics in animal feeding for safe food production. *Int J Food Microbiol* **141**(Suppl 1):S15-S28.

Gogineni VK, Morrow LE, Malesker MA (2013). Probiotics: Mechanisms of action and clinical applications. J *Prob Health* 1:1.

Gomez Arango LF, Barrett HL, Callaway LK, Nitert MD (2015). Probiotics and pregnancy. *Curr Diab Rep* **15**(1):567.

Gupta G (2011). Probiotics and periodontal health. *J Med Life***4**(4):387-394.

Haukioja A (2010). Probiotics and oral health. *Eur J Dent* **4**(3):348-355.

Homayouni A, Bastani P, Ziyadi S, Mohammad-Alizadeh-Charandabi S, et al (2014). Effects of probiotics on the recurrence of bacterial vaginosis: a review.*J Low Genit Tract Dis* **18**(1):79-86.

Issa IA , Moucari R (2014). Probiotics for antibiotic-associated diarrhea: Do we have a verdict? *World J Gastroenterol* **20**(47):17788-17795.

John GK, Mullin GE (2016). The gut microbiome and obesity. *Curr Oncol Rep* **18**(7)*:45.*

Kechagia M, Basoulis D, Konstatopoulo S, Dimitriadi D, *et al* (2013). *ISRN Nutr***2013**:481651.

Khoder G, Al-Menhali AA, Al-Yassir F, Karam SM (2016). Potential role of probiotics in the management of gastric ulcer. *Exp Ther Med* **12**(1):3-17.

Lee JE, Han JY, Choi JS, Ahn HK, *et al* (2012). Pregnancy outcome after exposure to the probiotic *Lactobacillus* in early pregnancy. *J Obstet Gynaecol* **32**(3):227-229.

Lee SJ, Cha J, Lee JW (2016). Probiotics prophylaxis in pyelonephritis infants with normal urinary tracts.

*World J Pediatr***12**(4):425-429.
Mackowiak PA (2013). Recycling Metchnikoff: probiotics, the intestinal microbiome and the quest for long life. *Front Public Heal***1**:52.
MaukonenJ,Saarela M (2015).Human gut microbiota :does diet matter?*Proc Nutr Soc***74**(1):23-36.
McFarland LV (2007). Meta-analysis of probiotics for the prevention of traveler's diarrhea. *Travel Med Infect Dis* **5**(2):97-105.
Meier R (2010). Probiotics in irritable bowel syndrome. *Ann Nutr Metab* **57**(Suppl 1): 12-13.
Meurman JH (2005). Probiotics: Do they have a role in oral medicine and dentistry?. *Eur J Oral Sci***113**(3):188-196.
Minemura M, Shimizu Y (2015). Gut microbiota and liver diseases. *World J Gastroenterol***2**(6):1691-1702.
Moraes-Filho JP, Quigley EM (2015). The intestinal microbiota and the role of probiotics in irritable bowel syndrome: a review. *Arq Gastroenterol* **52**(4):331-338.
Motevaseli E, Dianatpour A, Ghafouri-Fard S (2017).The role of probiotics in cancer treatment: Emphasis on their in vivo and in vitro anti-metastatic effects.*Int J Mol Cell Med***6**(2):66-76.
Niittynen L, Pitkäranta A, Korpela R (2012). Probiotics and otitis media in children.*Int J Pediatr Otorhinolaryngol***76**(4):465-470.
Onderdonk AB, Delaney ML, Fichorova RN (2016). The human microbiome during bacterial vaginosis. *Clin Microbiol* **29**(2):223-238.
Ouwehand AC (2016). A review of dose responses of probiotics in human studies. *Benef Microbes* **8**(2):143-151.
Park S, Bae JH (2015). Probiotics for weight loss: a systematic review and meta-analysis. *Nutr Res* **35**(7):566-575.
Parkes GC, Sanderson JD, Whelan K (2010). Treating irritable bowel syndrome with probiotics: the evidence. *Proc Nutr Soc* **69**(2):187-94.
Pavlović N, Stankov K, Mikov M (2012). Probiotics - Interactions with Bile Acids and Impact on Cholesterol Metabolism *Appl Biochem Biotechnol* **168**(7):1880-1895.
Raman M, Ambalam P, Kondepudi KK, Pithva S, *et al* (2013). Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. *Gut Microbes***4**(3):181-192.
Rashad A, Jing L, Xudong L, Miao J, Zhu B (2016). Human gut microbiota: the links with dementia development. *Protein Cell* **8**(2):1-13.
Rather IA, Bajpai VK, Kumar S, Lim J, Paek WK, Park YH (2016). Probiotics and atopic dermatitis: An overview. *Front Microbiol* **7**:507.
Redman MG, Ward EJ, Phillips RS (2014). The efficacy and safety of probiotics in people with cancer: A systematic review. *Ann Oncol* **25**(10):1919-1929.
Reid G (2006). Probiotics to prevent the need for, and augment the use of, antibiotics.*Can J Infect Dis Med Microbiol***17**(5):291-295.
Reid G (2016). Probiotics: Definition, scope and mechanisms of action. *Best Pract Res Clin Gastroenterol* **30**(1):17-25.
Roudsari MR, Karimi R, Sohrabvandi S, Mortazavian AM (2013). Health effects of probiotics on the skin. *Crit Rev Food Sci Nutr* **55**:1219-1240.
Saber A, Alipour B, Faghfoori Z, Yari Khosroushahi A (2016). Cellular and molecular effects of yeast probiotics on cancer. *Crit Rev Microbiol* **43**(1):96-115.
Sanaie S, Ebrahimi-Mameghani M, Mahmoodpoor A, Shadvar K,Golzari SE(2013).Effect of a probiotic preparation (VSL#3) on cardiovascular risk parameters in critically-ill patients. *J Cardiovasc Thorac Res***5**(2):67-70.
Sanchez M, Panahi S, Tremblay A (2015). Childhood obesity: A role for gut microbiota?. *Int J Environ Res Public Health***12**(1):162-175.
Sanders ME (2009). How do we know when something called 'Probiotic' is really a probiotic ? A guideline for consumers and health care professionals. *Funct Food Rev* **1**(1):3-12.
Sanders ME, Akkermans LM, Haller D, Hammerman C, *et al* (2010). Safety assessment of probiotics for human use. *Gut Microbes***1**(3):164-185.
Sharma BC, Singh J (2016). Probiotics in management of hepatic encephalopathy. *Metab Brain Dis* **31**(6): 1295-1301.
Sharma V, Garg S, Aggarwal S (2013). Probiotics and Liver disease. *Perm J* **17**(4):62-67.
Singh VP, Sharma J, Babu S, Rizwanulla, Singla A (2013). Role of probiotics in health and disease: a review. *J Pak Med Assoc* **63**(2):253-257.
Singhi SC, Kumar S (2016). Probiotics in critically ill children. *F1000Res* **5**:407 doi: 10.12688/f1000research.7630.1
Snydman DR (2008).The safety of probiotics. *Clin Infect Dis***46**(Suppl 2):S104-11.
Teitelbaum JE (2005). Probiotics and treatment of infectious diarrhea.*Pediatr Infect Dis J***24**:267-268.

Teixeira TFS, Grześkowiak KM, Salminen S, Laitinen K, Bressan J, Peluzio MCG (2013). Faecal levels of *Bifidobacterium* and *Clostridium coccoides* but not plasma lipopolysaccharide are inversely related to insulin and HOMA index in women. *Clin Nutr***32**:1017-1022.

Thompson JR (2016). Is irritable bowel syndrome an infectious disease?. *World J Gastroenterol* **22**(4):1331-1334.

Uccello M, Malaguarnera G, Basile F, D'agata V, *et al* (2012). Potential role of probiotics on colorectal cancer prevention. *BMC Surg* **12**(Suppl 1):S35.

Vandenplas Y, Huys G, Daube G (2015). Probiotics: An update. *Jornal de Pediatria***91**(1):6-21.

Vitali B, Cruciani F, Baldassarre ME, Capursi T, et al (2012). Dietary supplementation with probiotics during late pregnancy: outcome on vaginal microbiota and cytokine secretion. *BMC Microbiol***12**:236.

Vomero ND, Colpo E (2014). Nutritional care in peptic ulcer. *Arq Bras Cir Dig***27**(4): 298-302.

Wang Y, Kasper LH (2014). The role of microbiome in central nervous system disorders.*Brain Behav Immun* **38**:1-12.

Wickens K, Black PN, Stanley TV, Mitchell E,*et al*(2008).A differential effect of 2 probiotics in the prevention of eczema and atopy: A double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* **122**(4):788-794.

Zhang J, Abdullah JM (2013). The role of $GluA_1$ in central nervous system disorders. *Rev Neurosci* **24**(5): 499-505.z