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A Study On Probiotics And Their Use

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1) Abstract

The term “probiotic” was first used in 1965, by Lilly and Stillwell, to describe substances secreted by one organism which stimulate the growth of another. The use of antibiotics, immunosuppressive therapy and irradiation, amongst other means of treatment, may cause alterations in the composition and have an effect on the GIT flora. Therefore, the introduction of beneficial bacterial species to GI tract may be a very attractive option to re-establish the microbial equilibrium and prevent disease. Prebiotic is a non-digestible food ingredient that confers benefits on the host by selectively stimulating one bacterium or a group of bacteria in the colon with probiotic properties. Both probiotics and prebiotics are together called as Synbiotics. Various bacterial genera most commonly used in probiotic preparations are *Lactobacillus*, *Bifidobacterium*, *Escherichia*, *Enterococcus*, *Bacillus* and *Streptococcus*. Some fungal strains belonging to *Saccharomyces* have also been used. Probiotics have been shown to be effective in varied clinical conditionsranging from infantile diarrhoea, necrotizing enterocolitis, antibiotic-associated diarrhoea, relapsing *Clostridium difficle* colitis, *Helicobacter pylori* infections, inflammatory bowel disease to cancer, female uro-genital infection and surgical infections. *Lactobacillus rhamnosus* strain GG has proven beneficial affects on intestinal immunity. It increases the number of IgA and other immunoglobulins secreting cells in the intestinal mucosa. It also stimulates local release of interferons. It facilitates antigen transport to underlying lymphoid cells, which serves to increase antigen uptake in Peyer’s patches. Probiotics are live microorganisms, so it is possible that they may result in infection in the host.The risk and morbidity of sepsis due to probiotic bacteria should be weighed against the potential for sepsis due to more pathological bacteria and the morbidity of diseases for which probiotic bacteria are being used as therapeutic agents. Also, future, well-designed placebo controlled studies with validated results are required for ascertaining the true health benefits of probiotics The important point in this regard is careful selection of the probiotic agent, its dose standardization and a thorough knowledge of its beneficial effects

2) Key words : probiotics , lactobacillus, bifidobacterium, diarrhea

3) Introduction

The concept of probiotics probably dates back to 1908, when Noble Prize winner Eli Metchnikoff suggested that the long life of Bulgarian peasants resulted from their consumption of fermented milk products.[1] The term "probiotic" was first used in 1965, by Lilly and Stillwell for describing substances secreted by one organism which stimulate the growth of another.[2] Marteau *et al*, in 2002 defined them as "microbial preparations or components of microbial cells that have a beneficial effect on health and well being".[3]

Humans live in close association with vast numbers of micro-organisms present on the skin, in the mouth and in the gastro-intestinal tract. The greatest concentration of commensal organisms is found in the GI tract, which has more than 400 m2 of surface area. This constitutes the second largest surface area of the body after that of the respiratory tract. The GIT harbors a rich flora of than 500 different bacterial species, some of which have important health functions, which include stimulating the immune system, protecting the host from invading bacteria and viruses and aiding digestion,[4,5] The gut flora is acquire rapidly after birth, remains relatively stable throughout the life and is essential for human homeostasis. When the intestinal microflora is developing, the interactions between this microflora with the host results in evolution of a unique and distinct intestinal immune system.

The challenge facing this host mucosal immune system is to discriminate between pathogens and benign organisms by stimulating protective immunity without excessive inflammatory response that may disrupt the integrity of the GI mucosa,[5] The use of antibiotics, immunosuppressive therapy and irradiation, amongst other means of treatment, may cause alterations in the composition and have effect on the flora. Therefore, the introduction of beneficial bacterial species into the GI tract may be a very attractive option to reestablish the microbial equilibrium and prevent disease.[6]

4) Definition

The term 'probiotics' was derived from the Greek word, meaning "for life".[7] An expert panel commissioned by FAO (Food and Agriculture Organization) and WHO defined probiotic as "live micro-organisms," which, when administered in adequate amounts confers a health benefit on the host.[8] Various bacterial genera most commonly used in probiotic preparations are *Lactobacillus*, *Bifidobacterium*, *Escherichia*, *Enterococcus*, *Bacillus* and *Streptococcus*. Some fungal strains belonging to *Saccharomyces* have also been used [Table 1].[9-11] *Lactobacillus rhamnosus* GG (LGG) was the first probiotic, which received most clinical attention to date.[12]

The *Lactobacillus* strain used traditionally for fermentation by dairy industry was unable to implantthe gut, so,,*Lactobacillus rhamnosus* strain GG was discovered in 1985, by developing a list of ideal qualities for probiotics.[13] *Lactobacillus rhamnosus* strain GG has proven beneficial affects on intestinal immunity. It increasesthe number of IgA and other immunoglobulins secreting cells in the intestinal mucosa, stimulates local release of
interferons and facilitates antigen transport to underlying lymphoid cells, which serves to increase antigen uptake in Peyer's patches.[7]

Prebiotic is a non-digestible food ingredient that confers benefits on the host by selectively stimulating the growth and/or activity of one bacterium or a group of bacteria in the colon,and thus improve the host health.[14] Prebiotics are dietary carbohydrates that escape digestion in the upper gastrointestinal tract, alter the bacterial

composition of the gut, by changing the type of the substrate provided to the existing microbial population in the gut e.g. fructo oligosaccharides, gluco oligosaccharides and inulin. Both probiotics and prebiotics are together, Synbiotics, improves the survival of the bacteria in the GIT, so that their effect is more.

5) Properties of a probiotics

An ideal probiotic preparation should have the following properties [Table 2].[15] For adequate amount of health benefits, a dose of five billion colony forming units a day
(5x109 CFU/day has been recommended, for at least five days.[16]

The microorganisms used in probiotic preparations should be generally recognized as safe (GRAS), they should be resistant to bile, hydrochloric acid and pancreatic juice, have anti-carcinogenic activity and stimulate immunesystem, have reduced intestinal permeability, produce lactic acid, able to survive both acidic conditions of the stomach and alkaline conditions of the duodenum.[17]

Foods for human consumption that contain mainly lactic acid bacteria include fermented milks, cheeses, fruit juices, wine, and sausages. Single and mixed cultures of live microorganisms are used in probiotics preparation (18)

6) Criteria of an ideal microorganism as probiotic

1 High cell viability, thus they must be resistant to low pH and acids.

2 Ability to persist in the intestine even if the probiotic strain cannot colonize the gut..

3 Adhesion to the gut epithelium to cancel the flushing effects of peristalsis.

4 They should be able to interact or to send signals to the immune cells associated with the gut.

5 They should be of human origin.

6 Should be nonpathogenic.

7 Resistance to processing.

Table 1: Names of micro-organisms used as Probiotics[9-11]

Lactobacillus spa.	fibidobacterium spa	Streptococcus spa	Saccharomyces spa	Others
L. acidophilus	B.fibidum	S. thermophilus	S.boulardii	Bacillus cereus
L. casei	B.breve	S.salivarius subspp		Escheichia coli
L. fermentum	B.lactis			Enterococcus
L. gasseri	B.longum			propionibacterium
L. johnsonii	B. infantis			
L.lactis	B.adolescentis			
L.paracaei				

7) Mechanism of action

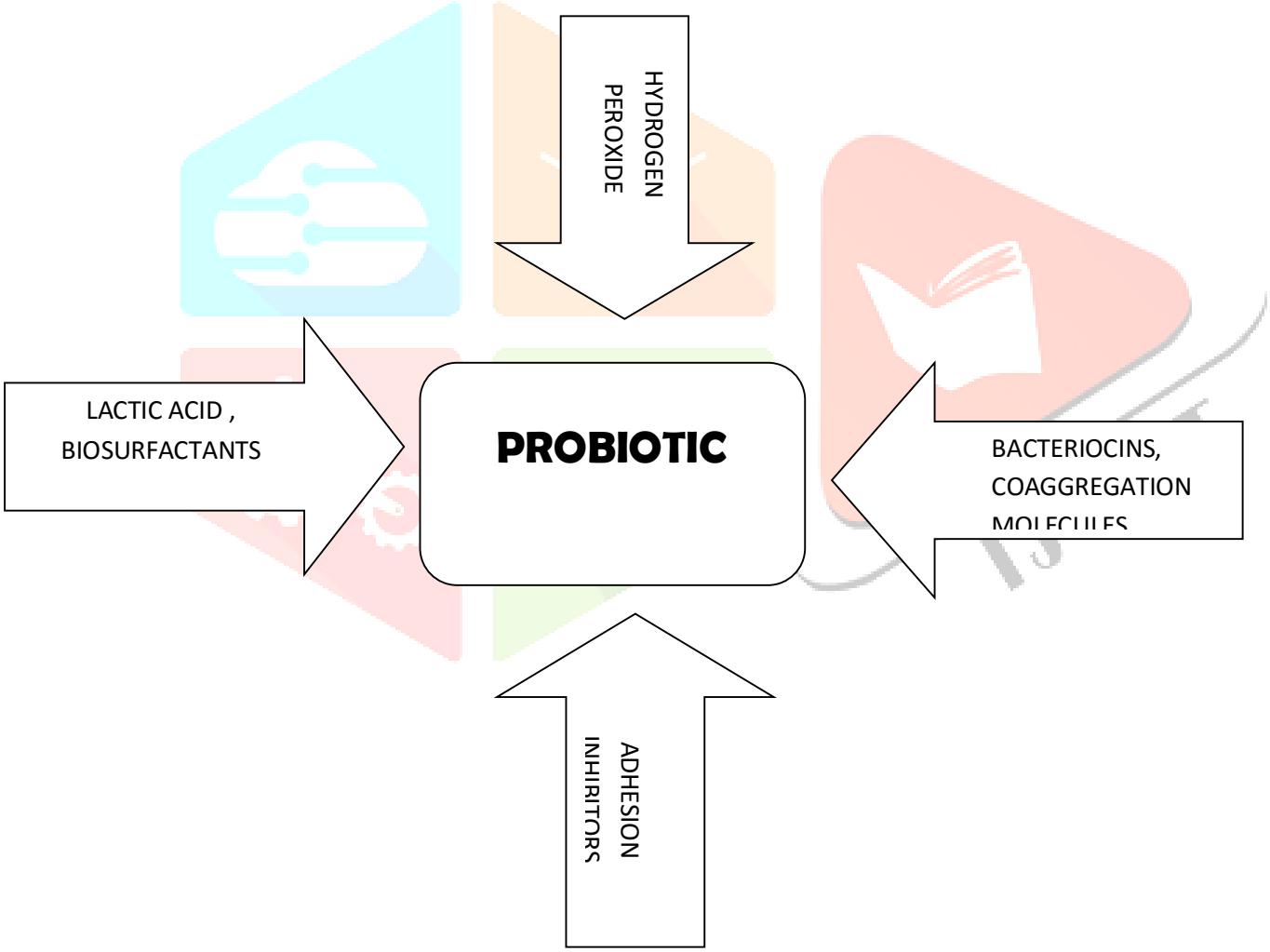
Several mechanisms have been postulated regarding action of Probiotics. Partial lactose digestion and been postulated as a possible mechanism against some types of diarrhoea. *Lactobacilli* used in the fermented milk industry have active beta-galactosidase to decrease the lactose concentration in dairy products, which may affect the severity of osmotic diarrhea due to organisms as rotavirus.[19.20] Lactic acid bacteria produce several metabolites like fatty free acids, hydrogen peroxide, bacteriocins etc. which prevent the growth of food borne pathogens in dairy products Figure.[21] Probiotics can also use enzymatic mechanisms to modify toxin receptors and block toxin mediated pathology.[22] Probiotic agents also prevent colonization of pathogens by competitiveinhibition.[23]

The other suggested mechanisms for the effect on intestinal microflora are lowering the intestinal pH,release of gut protective metabolites,regulation of intestinal motility and mucus production.
Gastrointestinal mucosa is the primary interface between the external environment and the immune system. Wheneverintestinal microflora reduces, antigen transport is increased indicating that the normal gut microflora maintains gutdefences.[24] The non-pathogenic probiotic bacteria interact with the gut epithelial cells and the immune cells to start the immune signals. These bacteria must interact with M cells in the Peyers patches, with gut epithelial cells, and with associated immune cells. Probiotic bacteria have been shown to modulate

immunoglobulin production. Secretory IgA plays an important role in mucosal immunity, contributing to the barrier against pathogenic bacteria and viruses. The increase in the number of IgA producing cells was the most remarkable property induced by probiotic organisms and also by fermented milk yogurt.[25,26]

Independent IgA induction has also been demonstrated.[27] The increase in profiles of certain cytokines (TNF-α IFN-γ, IL-10) has also been observed due to stimulation with probiotic bacteria.[28] The release of cytokines is induced to up or down regulate the immune responses and mainintestinal homeostasis. Interactions between probiotic micro-organisms and GALT(Gut associated lymphoid tissue), mechanisms of immunomodulation and anti-inflammatory properties are not yet fully understood.

8) Metabolic products of probiotic



9) Uses of Probiotics

Probiotics have been shown to be effective in varied clinical conditions- ranging from infantile diarrhoea, necrotizing enterocolitis, antibiotic associated diarrhoea, relapsing *Clostridium difficle* colitis, *Helicobacter pylori* infections, inflammatory bowel disease to cancer, female uro-genital infection and surgical infections.[7] *Evidences of probiotic effectiveness in Necrotising Enterocolitis* (NE) – NE is one devastating intestinal disorder that a preterm infant may face in a neonatal intensive care unit (NICU). It is characterized by abdominal distension, bilious vomiting, bloody diarrhoea, lethargy, apnoea, and bradycardia.[29] NE is reported in 10 to 25% of preterm infants, admitted to NICU and may affect 1/3 to1/2 of all low birth weight infants. The mortality ranges from 20 to 30% and those who survive have long term sequalae as short gut syndrome, intestinal obstruction and multi-organ failure.[30] Low birth weight pre-term infant delivered by Caesarean section often require intensive care and are breast fed only after several days. The normal process by which organisms such as *lactobacillus* species are ingested via
vaginal birth and propagated by mother's milk does not take place in these infants.[31]Therefore these infants are exposed to a plethora of pathogenic microbes like – *Clostridium, Escherichia, Salmonella, Shigella, Campylobacter, Pseudomonas, Streptococcus, Enterococcus, Staphylococcus* and coagulase negative *Staphylococcus*, which colonize the intestine and increase the risk of NE..Further, pre-term infants, given formula feeding have less *Lactobacillus* and *Bifidobacterium* species in their stool compared to controls. These findings suggest a correlation between NE and*Lactobacillus* species.

A human trial with 2.5 x 108 live *LactobacilluS acidophilus* and 2.5 x 108 live *Bifidobacterium infantiS* given to 1237 newborn in Columbia, resulted in 60 reduction in NE and overall mortality.[32] A correlation between normal gut microflora and protection agains various infections has been reported. This supports the concept of early intestinal colonization with organisms such as *Lactobacillus rhamnosus* and *Bifidobacterium infantis* and subsequent protection against NE.[33]

1o) Diarrhea

Probiotics have preventive as well as curative effects on several types of diarrhoea of different etiologies. Prevention and therapy of diarrhoea have been successfully investigated for numerous dietary probiotics micro-organisms (eg. *Lactobacillus rhamnosus* GG, *L. reuteri,* certain strains of *L. casei, L. acidophilus, Escherichia coli* strain Nissle 1917 and certain *Bifidobacteria* and *Enterococci* (*Enterococcus faecium* SF
68) as well as the probiotic yeast *Sacchromyces boulardii* have been investigated with regards to their medicinal use, either as single strain or as mixed culture probiotics.[34]

10.1) Rota virus diarrhea

–Various randomized, double blinded and placebo controlled studies have shown
beneficial effects with *Lactobacillus rhamnosus* strainGG and *Bifidobacterium lactis* BB-12 for prevention and *Lactobacillus reuteri* SD 2222 for treatment of acute diarrhea caused by rota virus in children.[26,35,36]

10.2) Antibiotic associated diarrhea

–Although newer antibiotics with a broad spectrum of activity and fewer side effects have been developed, the incidence of antibiotic associated diarrhea (AAD) still ranges from 3.2-29/100 hospitalized patients.[37] The complications of AAD include electrolyte imbalance, dehydration, pseudomembrane colitis and toxic

megacolon. Antibiotics with a spectrum of activity that includes anaerobic bacteria (esp. cephalosporins, penicillin or clindamycin) have been associated with higher rates of AAD, although nearly all types of antibiotics have been associated. A meta analysis to evaluate the efficacy of probiotics in prevention and treatment of AAD showed an Odds ratio of 0.39 (p less than 0.001) in favor of active treatment over placebo with *Saccharomyces boulardii.*[18]

10.3) Radiation induced diarrhea

–A double blind, placebo controlled trial was done to investigate the efficacy of a high potency probiotic preparation on prevention of radiation – induced diarrhoea in cancer patients. About 490 patients, who underwent adjuvant postoperative radiation therapy, were given either high potency probiotic preparation VSL#3 or placebo. Efficacy end points were incidence and severity of radiation-induced diarrhoea and daily number of bowel movements. Results were- more placebo patients had radiation induced diarrhoea than VSL #3 patients and more patients given placebo suffered grade 3 or 4 diarrhoea compared with VSL #3 recipients. So it was concluded that,probiotic lactic acid producing bacteria are an easy, safe and feasible approach to protect cancer patients against the risk of radiation.[38]

10.4) Traveller's diarrhea

–Traveler's diarrhoea is a common health complaint among travelers. Rates of traveler's diarrhoea can range from five to 50% depending upon destination. A meta-analysis was done on published randomized controlled clinical trials of traveler's diarrhea cases. It was concluded that probiotics significantly prevent traveler's diarrhoea. *Saccharomyces boulardii* and a mixture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* had significant efficacy[39

11) Helicobacter pylori

H. pylori, is a major cause of chronic gastritis and peptic ulcer and a risk factor for gastric malignancies. Antibiotics based *H. pylori* eradication treatment is 90% effective. However, it is expensive and causes side effects and antibiotic resistance. A literature search of MEDLINE database (1966-2006) was performed on studies dealing with *H. pylori* and probiotics. The studies revealed that Probiotics had an *in vitro* inhibitory effect, reduced *H. pylori* associated gastric inflammation in animals, improved *H. pylori* associated gastritis and also probiotic treatment reduced *H. pylori* therapy associated side effects.[40]

12) Inflammatory bowel disease

Inflammatory bowel disease classically includes ulcerative colitis and Crohn's disease representing different patterns of chronic inflammation of GIT. Recent clinical and experimental observation implicates an imbalance in the intestinal mucosa with relative predominance of aggressive bacteria and relative paucity of protective bacteria[41]
and also stimulation of proinflammatory immunological mechanisms.[34] Various preliminaries studies suggest a positive response to probiotics in patients with IBD, causing decreased expression of inflammatory markers *ex-vivo*,[42] increasing the immune response[43] and improving the gut barrier functions.[44] Thus, probiotics have a potential for inducing or maintaining remissions in IBD. However, further studies are required to have a proven beneficial role in IBD cases. Limited data from small controlled studies would suggest that VSL#3 is a reasonable therapy in the primary and secondary type of pouchitis. [45, 46]

13) Cancers

In intestinal tumors, prevention or delay of tumor development by lactobacilli is that they bind to mutagenic compounds in the intestine and also suppress the growth of bacteria which convert procarcinogens into carcinogens.[47] The ability of lactobacilli to reduce the risk of cancers has also been suggested based on their ability to modify gut microflora and to decrease β-glucoronidase and, other carcinogen levels.[48] Studies indicate that recurrences of urinary bladder cancers appears to decrease by internal instillation of probiotics like *L. casei* Shirota, but this finding needs confirmation.[49]

14) Surgical Infections

Before the advent of antiseptics and antibiotics, fermented milk was used for healing wounds and to fight infections. Recent studies show some success in application of probiotics for treating and preventing surgical infections. Studies shows *L. fermentum* RC-14 was shown to significantly inhibit *S. aureus* infection and bacterial adherence to surgical implants also.[50with oat fibres for one week had significantly fewer episodes,of infection and pancreatic abscesses. Also, these studies indicate the role of probiotics to decontaminate the intestine prior to gut surgery instead of antibiotics. [51]]

15) Other Benefits

Role in uro-genital infections- An abnormal microbiota of the vagina predisposes a female to symptomatic vaginal or bladder infection. Two strains, *Lactobacillus GG*(ATCC 53103) and *Lactobacillus rhamnosus* GR-1 appear to be effective at colonizing and protecting urogenital tract.[52,53] The various by-products of lactobacillus metabolism that have an antagonistic effect against urinary and vaginal pathogens are biosurfactants that inhibit adhesion; the acids,bacteriocins and hydrogen peroxide inhibit growth;and the coaggregation molecules block the spread of the pathogens.[54] In this direction, the administration of *Lactobacillus rhamnosus GR-1* and *L.fermentum RC-14* as a self therapy by mouth to restore and maintain urogenital health is a major step for prevention and treatment of uro-genital infections.[55] By reducing the risk of bacterial vaginosis, probiotics may also help to reduce infant mortality and pre-term labour in pregnant women.

16) Role in prevention of transmission of AIDS and STD

- *Lactobacilli* play a critical role in the regulation of the vaginal microflora. It has been suggested that the production of H2O2 rather than a particular species of *Lactobacillus*, is
essential in the regulation of the vaginal flora. This toxic molecule is the most potent local microbicide present in the human vagina. The findings of experiments have suggested that LB+ given at high concentrations is viricidal for HIV- 1.[56,57] There is also an inverse association between vaginal *Lactobacilli* and HIV seroconversion.[58] These studies suggest that LB+ may play a role in protecting women against some pathogens in the vagina. Role in infection control programs and eradication of multi-drug resistant micro-organisms: - The alarmingincrease of inappropriate antibiotic use and bacterial resistance, along with renewed interest in ecological methods to prevent infections, makes probiotics a very interesting field for research. A case report describes a 68-year-old woman from Japan with a decubitus ulcer colonized by methicillin resistant S*taphylococcus aureus* who was successfully treated with a *Lactobacillus* preparation.[59] Studies of this potential use may haveprofound impact in coming years.

17) Antibacterial effects

In vitro studies suggest multiple specific activities of different probiotic agents against several pathogens, including *Listeria monocytogenes*,[60] *Salmonell typhimurium*,[61] *E. coli*[62,23] and *H. pylori*[63] amongothers. Therefore, probiotic agents may provid prototypic antimicrobial substances that will be useful for pharmaceutical companies in the development of new Antibiotics. Role In oral candidiasis -None of probiotic bacterial species completely prevent mucosal candidiasis, but *B. animalis* was found to reduce the incidence and severity of mucosal candidiasis. Probiotic bacteria also modulated antibody and cell mediated immune responses to *C. albicans*. Thus the study demonstrated that probiotic bacteria have biotherapeutic potential in prophylaxis and therapy of *C. albicans* infections by a variety of immunologic and non-immunologic mechanisms.[64]

18) Probiotics in critical illness

Some studies propose that probiotics have an important emerging role in managing critical illnesses originating in gastrointestinal tract like acute pancreatitis. In cirrhotic patients, probiotics have shown a decrease in incidence of encephalopathy. Also reduction of post liver transplant infective complications using probiotics have been seen.[65] It also helps in colonic involvement in Stevens –Johnson syndrome.[66) Probiotic *Lactobacillus reuteri* reduces gingivitis and decreases gum bleeding.[67]

19) Probiotics in allergic diseases

Most studies on the use of probiotics have assessed patients with atopic eczema. Also work has been carried out on the role of probiotics in respiratory allergies like asthma.[68] Experimental studies suggest that specific strains of probiotics may act upon the intestinal mucosa with potential modulation of the allergic response.[69 Probiotics sometimes relieve the symptoms of anxiety.[17] Lactic acid produced by *Lactobacillus* can be used as food preservative, flavouring agent and emulsifier.[23]

20) Safety

Probiotics are live micro-organisms and hence, I it is possible that they may result in infection in the host Different strains of probiotics have different safety profiles Although probiotic therapy is generally considered safe, the concept of willingly ingesting live bacteria remains somewhat counter intuitive. Systemic infection has rarely been reported with *Bifidobacterium*, although many cases of sepsis secondary to *Lactobacillus rhamnosus* GG or *Lactobacillus casei* have been reported.[70,71] The issue of safety becomes more complex when the organism is *Enterococcus* spp. As probiotic.[72)

The risk and morbidity of sepsis due to probiotic bacteria should be weighed against the potential for sepsis due to more pathological bacteria and the morbidity of the diseases for which probiotic bacteria are being used as therapeutic agents. The reports of sepsis are mainly seen in immunocompromised or infants.[70,71] Another study done on dietetic products for infants suggests that caution should be exerted regarding probiotic therapeutics and use of other organisms than *Lactobacillus* should be encouraged.[73] But the conclusion based on different reports is that the risk of infection with probiotics *Lactobacilli* or *Bifidobacterium* is similar to infection with commensal strains, and that consumption of such products presents a negligible risk to consumers including immunocompromised hosts.[74] However ,in order to establish safety guidelines for probiotic organisms, FAO and WHO recommends that probiotic strains be characterized at a minimum with a series of tests, like antibiotic resistance patterns, metabolic activities, toxin production, hemolytic activities, infectivity in immunocompromised animal models, side effects in humans, and adverse outcome in consumers.[75] FAO/WHO developed Operating Standards in 2002, which gave guidelines for all companies producing probiotic products

. These guidelines include

1) Implementation of guidelines for use of probiotics;
2) Phase I, II and III clinical trials to prove health benefits that are as good as or better than standard prevention or treatments for a particular condition or disease;
3) Good manufacturing practice and production of high quality products;
4) studies to identify mechanism of action in-vivo;
5) Informative/precise labeling;
6) development of probiotic organism that can carry vaccines to hosts and /or antiviral probiotics;

7) Expansion of proven strains to benefit the oral cavity, nasopharynx, respiratory tract, stomach, vagina, bladder and skin as well as for cancer, allergies and recovery from surgery/injury.[76]

21) Conclusion

Probiotic therapy has already made its way in the treatment of number of conditions-Infectious, inflammatory, neoplastic and allergic. There is a long list of potentials of giving probiotics in a number of these conditions. But before bringing probiotics into routine usage, proper evaluation of these products is essential. Several important criteria and standards regarding quality and reliability have to be met. Thus future well designed placebo controlled studies with validated results are required for ascertaining the true health benefits of these products. The important point is careful selection of the probiotic agent, its dose standardization and a thorough knowledge of its beneficial effects over and above the toxic effects, so that this traditional therapy proves to be an effective tool for medical therapy

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