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Probiotics: An Approach for Better Treatment

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ABSTRACT

Probiotics are live nonpathogenic preparation administered to improve and restore the microbial balance of gastrointestinal tract. These preparations exert their action through various mechanisms, including decreasing the invasion and colonization of pathogenic organism, modifying the host immune responses, lowering the intestinal pH, enhancing the barrier functions, detoxification of ingested carcinogens and by producing the beneficial metabolic products like antimicrobial substances, enzymes and peptides. The recent trends of rationale use of xenobiotics and chemotherapeutic agents against various pathological conditions, lead the imbalance of normal GIT flora, which result the development of drug resistance and other opportunistic infections. The administrations of probiotics along with dietary supplements to GI tract may be a very attractive option to re-established the microbial equilibrium and improve the host welfare. This review mainly focuses to communicate the possible clinical application of probiotics as to minimize the frequent use of xenobiotics and chemotherapeutic agents.

Key words: Probiotics, Microbial flora, Immunomodulation, Antimicrobial activity.

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INTRODUCTION

In the present scenario, xenobiotics and chemotherapeutic agent are the first line therapy choice by most of physicians in treatment of infections and intestinal disorders. However, irrational and overuse of these agents is directly associated with Superinfections (Clindamycin associated pseudomembranous colitis), perturbation of balance of normal intestinal flora (Ampicillin associated diarrhea), risk of increased incidence of drug resistance (methicillin resistant Staphylococcus aureus and VRSA) and other opportunistic infections (hospital acquired infections by pseudomonas aeruginosa). The previously mentioned complications can be avoided, if the uses of the chemotherapeutic agents minimized and specific agent has to be used against specific pathogens.

Within the normal bacterial flora, host defense ensured through a balance between non-pathogenic commensals and pathogenic bacteria. However, some of these microorganisms possess pathogenic potential and take advantage of certain gastrointestinal environmental opportunity, colonizing the GIT and causing different intestinal disorders. Indeed, the commensals microbiota is quantitatively and qualitatively unbalanced in a wide range of intestinal and autoimmune disorders [1, 2**]**. Imbalance of this microbial flora results other pathological complication shown in **fig. 1.**

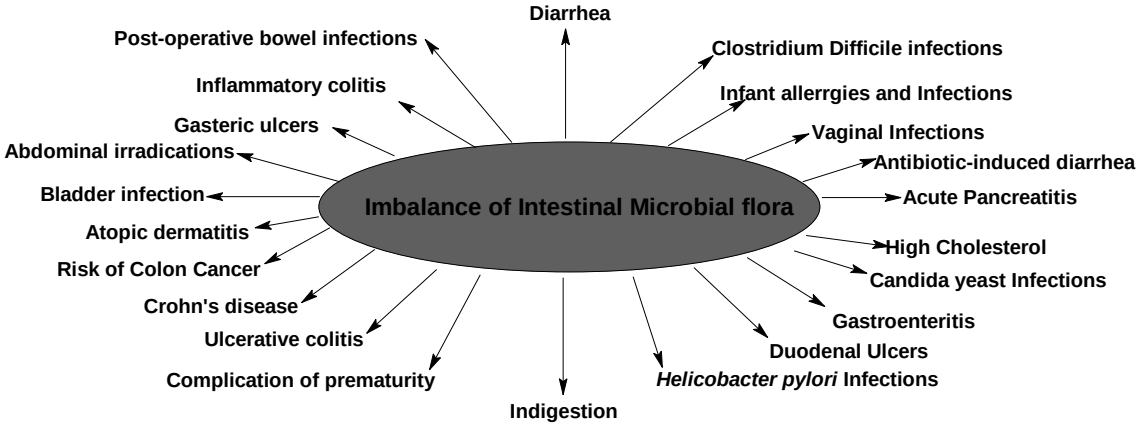

Figure 1: Imbalance of intestinal Microbial flora leads following complications.

The administration of nonpathogenic microorganism or microbial mixtures or component of bacterial cells along with dietary supplements to GI tract may beneficial option to maintain the intestinal homeostasis [2**]**. Such preparations called probiotics. The term 'probiotics' was derived from the Greek word, meaning "for life". An expert panel commissioned by FAO (Food and Agriculture Organization) and WHO defined probiotic as Live microorganism, which, when administrated in adequate amounts confers a health benefit on the Host [5]. Various bacterial genera most commonly used in probiotic preparation are Lactobacillus, Bifidobacterium, Escherichia, Entrococcus, Bacillus and Streptococcus. Some

fungal Strain belonging to Scaccharomyces have also been used [4]. List of bacterial strain along with their established effects summarized in **table 2.**

These probiotic microorganisms appear to be promising candidates for the treatment of various clinical conditions in both humans and animals. Now, probiotics are found to put forth health advantages such as improving lactose intolerance, modulate immunity, lower serum cholesterol, treat rheumatoid arthritis, prevent cancer, improve lactose intolerance, prevent or reduce the effects of atopic dermatitis, diarrhea and constipation, candidiasis, urinary tract infections [3, 4**]**, as well as bioconversion of bioactive peptides for antihypertensive effect and reducing serum cholesterol level [4]. The spectrum of beneficial effects of probiotic preparations summarized in **figure 1.** This review mainly focuses to communicate and explore the possible clinical application of probiotics.

Microbial Flora

The gastrointestinal tract of a fetus is sterile. During birth and thereafter, microbes from mother and the surrounding environment colonize the infant gut. Babies born of vaginal deliveries acquire the bacterial strains from mother's anogenital area whereas babies born by caesarean section are mainly acquiring the bacterial strains from surrounding. After birth, environmental as well as mothers oral and cutaneous bacteria transferred to infant. Initially large numbers of E. coli and streptococci colonizes infant's guts. Breast fed babies become dominated by bifidobacteria as possibly breast milk contains growth factors for these anaerobes. The microflora of formula fed infants is more diverse and dominated by enterobacteriaceae, enterococci, bacteroids etc. After ingestion and introduction of solid food, the micro flora of breast-fed infant becomes similar to formula-fed infant. By the end of second year of life, the fecal flora resembles that of normal adults [6].Common micro flora of Gut consists of Anaerobes: Bifidobacterium, Clostridium, Bacteroides, Eubacterium and Aerobes: Escheichia, Enterococcus, Streptococcus, Klebsiella. [7, 9]

The composition of the GI tract flora varies between individuals and also within the same individual during life. Various factors such as diet, climate, ageing, medication (particularly antibiotic consumption), illness, stress, and lifestyle can upset this balance leading to diarrhea, mucosal inflammation, or other serious illnesses. Maintenance of an optimal gut flora balance requires that 'friendly' bacteria, such as the Gram-positive lactobacilli and bifidobacteria dominate (>85% of total bacteria), form a barrier to pathogenic bacteria [8].

Physiological Function of Microbial Flora

The bacterial flora of the skin and mucosal surfaces is an important barrier to infection. In the recent years, its has been confirmed that normal flora of GIT not only play a vital role in digestion, absorption of nutrients and excretion of the waste products, it also fulfill many other function, which are essential to our well being [10]. Major functions of the gut microbiota include metabolic activities that result in salvage of energy and absorbable nutrients, tropic

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effects on the intestinal epithelium and protection of the host against invasion by harmful microbes [11, 12]. The other potential established physiological functions of microbial flora summarized in **table 1.**

Table 1: Examples of Bacterial Specific Adherence to host cells or tissues.

S.No.	Bacterium	Bacterial adhesin	Attachment site
1	Streptococcus pyogenes	Cell-bound protein (M-protein)	Pharyngeal epithelium
2	Streptococcus mutans	Cell- bound protein (Glycosyl transferase)	Pellicle of tooth
3	Streptococcus salivarius	Lipoteichoic acid	Buccal epithelium of tongue
4	Streptococcus pneumoniae	Cell-bound protein (choline-binding protein)	Mucosal epithelium
5	Staphylococcus aureus	Cell-bound protein	Mucosal epithelium
6	Neisseria gonorrhoeae	N-methylphenyl- alanine pili	Urethral/cervical epithelium
7	Enterotoxigenic E. coli	Type-1 fimbriae	Intestinal epithelium
8	Uropathogenic E. coli	P-pili (pap)	Upper urinary tract
9	Bordetella pertussis	Fimbriae ("filamentous hemagglutinin")	Respiratory epithelium
10	Vibrio cholerae	N-methylphenylalanine pili	Intestinal epithelium
11	Treponema pallidum	Peptide in outer membrane	Mucosal epithelium
12	Mycoplasma	Membrane protein	Respiratory epithelium
13	Chlamydia	Unknown	Conjunctival or urethral epithelium

Table 2: Potential and established effects of probiotic strains.

S.No.	Target health benefit	Postulated mechanism
1	Aid lactose digestion	Bacterial lactase hydrolysis lactose
2	Resistance to enteric pathogens	• Secretory immune effect • Colonization resistance • Alteration of intestinal conditions to be less favorable for pathogenicity (pH, short chain fatty acids, bacteriocins) • Alteration of toxin binding sites • Influence on gut flora populations • Adherence to intestinal mucosa, interfering with pathogen adherence • Upregulation of intestinal mucin production, interfering with pathogen attachment to intestinal epithelial cells
3	Anti-colon cancer effect	• Mutagen binding • Carcinogen deactivation • Inhibition of carcinogen-producing enzymes of colonic microbes • Immune response • Influence on secondary bile salt concentration
4	Small bowel bacterial overgrowth	• Influence on activity of overgrowth flora, decreasing toxic metabolite production • Alteration of intestinal conditions to be less favorable to overgrowth flora activities or populations
5	Immune system modulation	• Strengthening of non-specific defense against infection and tumors • Adjuvant effect in antigen-specific immune responses • Enhancement of secretory IgA production
6	Allergy	• Prevention of antigen translocation into blood stream

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7	Blood lipids, heart disease	• Assimilation of cholesterol within bacterial cell • Increased excretion of bile salts due to deconjugation by bile salt hydrolase • Antioxidative effect
8	Antihypertensive effect	• Peptidase action on milk protein yields tripeptides which inhibit angiotensin 1 converting enzyme • Cell wall components act as angiotensin converting enzyme inhibitors
9	Urogenital infections	• Adhesion to urinary and vaginal tract cells • Colonization resistance • Inhibitor production (H2O2, biosurfactants)
10	Infection caused by Helicobacter pylori	• Production of inhibitors of H. pylori (lactic acid and others)
11	Hepatic encephalopathy	• Inhibition of urease-producing gut flora

Table 3: List of Bacterial strain used as probiotics with therapeutic importance

Bacterial strain	Therapeutic importance
Lactobacillus Bacteria(DDS-1 strain**)**	• Stimulate the immune system, increasing levels of interleukin-1 alpha (IL-1 alpha) and tumor necrosis factor-alpha (TNF-alpha). **[83]**. • Alleviates lactose intolerance by producing significant amounts of the lactose digesting enzyme lactase, • Inhibits gastrointestinal pathogens by producing antimicrobial substances such as acidophilin.
Lactobacillus rhamnosus GG strain	• Effective treatment for diarrhea caused by Clostridium difficile infection **[84]**.. • In preventing allergic illness **[85]**.
Lactobacillus bulgaricus	• Enhancing the digestibility of milk products and other proteins • Producing natural antibiotic • Suppress inflammatory immune reactions in the intestinal wall thus preventing tissue damage**[86]**.. • Stimulate activity in part of the gut immune system called the Peyer's patches which provide defense against pathogenic organisms within the gut**[87]**..
Lactobacillus salivarius	• It produces large amounts of lactic acid that completely inhibits the growth of H. pylori and reduces the associated inflammatory response. **[88]**. • The first bacteriocin (natural antibiotic substance) to be isolated and studied at the genetic level was taken from a strain of L. salivarius **[89]**..
Lactobacillus plantarum	• Important player in antimicrobial defense and is effective against both extra and intracellular pathogens. • Capable of digesting semi-digestible fibres such as those found in onions, garlic, wheat, oats, rye and yeast. • Ability to break down bile acids and lower cholesterol **[90]**.

Lactobacillus casei	• Inhibit the growth of the peptic ulcer causing bacteria H. pylori **[91]**.. • L. casei (combined with FOS), when given along with a re-nutrition diet, enhanced the immune response and increased resistance to certain pathogenic bacteria in the digestive tract **[92]**.
Lactobacillus sporogenes	• Able to lower cholesterol levels by 104 points. • In reducing the number of episodes and duration of diarrhea following antibiotic treatment in children **[93]**. • In the treatment of gut dysbiosis, • Vaginitis and Aphthous stomatitis**[94]**.
Bifidobacteria bifidum	• Reduces the inflammatory response in the colon • Stimulates the body's fluid immunity • Anti-oxidant action and was able to protect the intestinal lining from lipid peroxidation in iron overloaded mice [95].
Bifidobacteria longum	• Able to eliminate the nitrates commonly found in foods. • Inhibit the action of vero cytotoxin produced by some strains of E.coli, which can cause hemorrhagic colitis and hemolytic uremic syndrome in humans **[96]**. • Protective effect against infection with Salmonella Typhimurium, • An anti-inflammatory action **[97]**.
Bifidobacteria infantis	• Inhibitory action on invasive pathogenic bacteria such as E.coli. • Inflammatory bowel disease (IBD) caused by organisms called bacteroides **[98]**. • Diarrhea..
Streptococcus thermophilus	• Have powerful antioxidant activity, • Anti-tumour activity that is especially effective against colon cancer cells.
Homeostatic Soil Organisms (HSO's)	• Aggressively killing pathogens, • Producing specific antigens that act to stimulate the immune system, • Create superoxide dismutase (SOD) a powerful antioxidant enzyme • Metabolize proteins and eliminate toxins.

Colonization of probiotics

The colonization of probiotic strain is a tissue specific process, which depends on the tissue specificity of host and the bacterium. The establishment of probiotic strains involved, tissue tropism, specific adherence and biofilm formation by the bacterium.

Tissue tropism: It is developed when the host provides essential nutrients, oxygen, pH and temperature for the normal existence bacterium. For example, lactobacillus acidophilus (Doderlein's bacilli), an acid-tolerant lactobacilli, as to fulfill their requirement of glucose, they ferment the available glycogen of vaginal epithelium into lactic acid and maintained the pH of vagina and cervix between 4.4 and 4.6.

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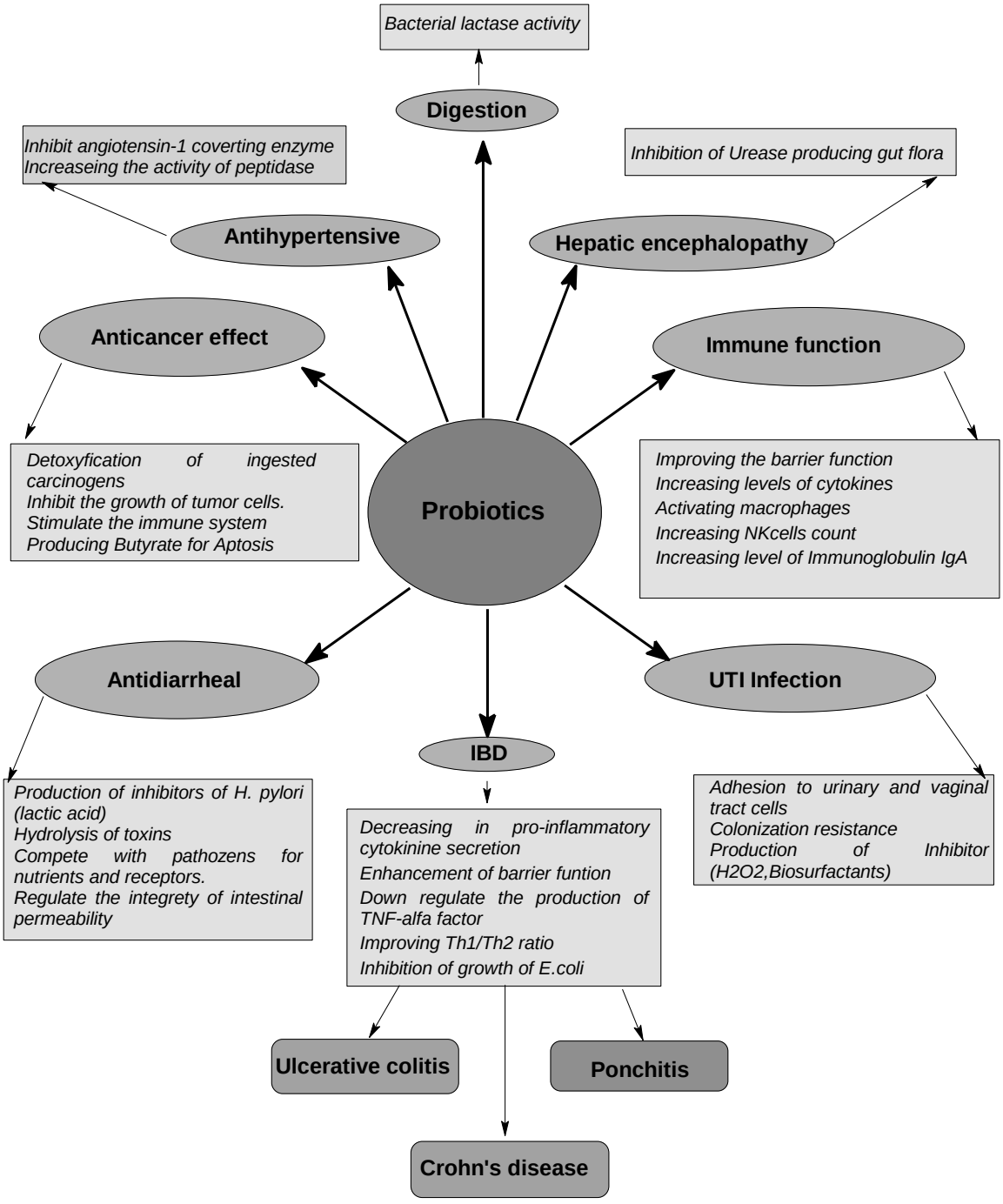

Figure 2: Clinical Importance of probiotics

Specific adherence: It involves biochemical interactions between bacterial components (ligands or adhesins) and host cell molecular receptors (Toll receptor). The bacterial components that provide adhesins are molecular parts of their capsules, fimbriae, pili, or cell walls. The receptors on human cells or tissues are usually glycoprotein molecules located on the host cell or tissue surface [14]. Examples of some bacterial specific adhesion/ receptor listed in **table 1.**

Biofilm formation: Some of the indigenous bacteria are able to construct biofilms on a tissue surface, or they are able to colonize a biofilm built by another bacterial species [15]. The classic example of biofilm formation is plaque, naturally constructed a biofilm on which consortia of bacteria may reach a thickness of 300-500 cells on teeth surfaces.

Mechanism of Action of Probiotics

Probiotics exert several beneficial effects on human health, including interaction with the immune system, production of antimicrobial substances, enhancement of the mucosal barrier function and competition with enteropathogens for adhesion sites [13, 15]. Probiotic bacteria may put forth their beneficial roles in several ways. Some might produce antimicrobial substances; some compete with pathogenic bacteria for nutrients or for binding sites on the intestinal wall and some modulate the host immune system. Whatever the mechanism, for favorable effects to occur and last it is necessary to consume live probiotic bacteria regularly as they are only transient in the intestinal tract and do not become part of the host's gut microflora. The various working mechanism of the probiotics discussed in fig. 2, table 2, and table 3.

Probiotic in Immune Function.

It is well established that Probiotics influence the several components of an immune response including humoral, cellular or innate immunity. Recently, it has been shown that specific probiotic strains can influence the secretion of cytokines and help to direct native helper T cells towards either a Th1 dominant for cell-mediated immune response or towards a Th2 dominant for humoral immune response [16].

Th 1 cell pathway

The Th1 cell supports cell-mediated immunity, preventing disease from intracellular pathogens like viruses, certain bacteria, yeast, fungi and protozoans. Th1 cell associated immunity also has a major role in preventing tumor cell development. Same time over stimulation of Th1 cells cause production of large amount of pro-inflammatory cytokines like IFN-γ and TNF-α, which ultimately results the inflammation of gastrointestinal tract associated with pathologies of like Crohn's disease [17], H. pylori gastritis, cellular autoimmunity, chronic recurrent inflammation and possibly rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus [16]. Probiotic strains can help to down regulate these Th1 dominant disorders and return the balance between Th1/Th2 immune response. For example, TNF-α plays a key role in the pathogenesis of intestinal inflammation in Crohn's disease, a disease associated with Th1stimulatione. In a study, Borruel et al. (2002), observed a significant reduction in the production of TNF-α by inflamed Crohn's disease mucosa when cultured with L. casei or L. bulgaricus, but not with L. crispatus or E. coli. [18].

The authors accomplished that these probiotic strains had the ability to attenuate the release of the pro-inflammatory cytokines that promote Th1 rigidity by interacting with the intestinal mucosal immunocompetent cells [16].

Th 2 cell pathway

The Th2 cell pathway supports humoral immunity by activating specific B cells. Th2 cells, release the cytokines IL-4, IL-13, IL-5 and IL-10 that activate antibody formation or release from immune cells like B cells, mast cells or eosinophils. Persistent, uncontrolled Th2 activation is associated with diseases like atopy (including allergies, eczema, asthma, and allergic rhinitis), chronic fatigue and immunodeficiency syndrome, eosinophilic rhinosinusitis, ulcerative colitis and possibly certain cancers [16]. In a study it has been observed that ingestion of L. rhamnosus GG by breast –feeding mothers and new born babies resulted a 50% can reduction in the risk of developing atopic eczema in babies [19].

The studies provide an example of specific probiotic strain is a better alternative to down regulate the Th1dominance associated with GIT inflammatory disorders and Th2 dominance associated with the development of allergies.

Role of extracellular protein of probiotic strain

It has been reported that probiotics bacteria have tendency to liberate proteins (extracellular proteins) as the metabolic product, could diffuse through the mucus layer and interact with epithelial and immune cells. They stimulate the several kinases, such as mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI-3K) and glycogen synthase kinase-3 (GSK-3) [20] , which finally triggers their signaling pathway and cellular response, including secretion of autocoids (chemokines, cytokines antibacterial peptides -defensins), mucus secretion, rearrangement of tight junction and modulation of immune function of gut-associated lymphoid tissue (GALT) cells [21-22].

Serine protease inhibitor (Serpin) from Bifidobacterium longum subsp. longum NCC2705 was the first extracellular protein shown to interact directly with the host factors. Serpin competently inhibits pancreatic and neutrophil elastases, which are recruited in the intestinal mucosa from the blood vessels on the influence of inflammatory cytokines, and therefore are involved in inflammatory episodes [22].

L. acidophilus NCFM cells has been shown to released the S-layer protein A (SlpA) which induce the production of IL-10 in dendrite cells. The effects of SlpA on DCs involved a direct interaction of SlpA with the surface lectin DC-SIGN. This result suggests a direct role for SlpA as an anti-inflammatory protein [24].

Yan and co-workers have shown that both p40 and p75 are important extracellular proteins for GIT homeostasis, involved in both cell proliferation and apoptosis, and in the maintenance of the mucosal barrier [25].

E. coli Nissle 1917 secretes proteinaceous molecules capable of blocking the adhesion and colonization of the intestinal cell line INT407 by Salmonella sp., Yersinia enterocolitica, Shigella flexneri, Legionella pneumophila and Listeria monocytogenes [26].

Antiallergic effect of probiotics

Exposure to microbial flora early in life allows for a change in the Th1/Th2 balance, favoring a Th1 cell response. Allergic disorders are associated with a shift of the Th1/Th2 cytokine balance towards a Th2 response. This leads to activation of Th2 cytokines and the release of interleukin-4 (IL-4), IL-5, and IL-13 as well as IgE production. Probiotics can potentially transform the toll-like receptors and the proteoglycan recognition proteins of enterocytes, leading to activation of dendritic cells and a Th1 response. The resulting stimulation of Th1 cytokines can suppressTh2 responses [27].

Pediatric studies suggest that probiotic use in children with atopic conditions such as atopic dermatitis results in enhancement of IFN-production and decreased IgE and antigen-induced TNF-α, IL-5, and IL-10secretion [28-29]. The frequency of IgE-associated dermatitis rather than other types of atopic dermatitis was decreased after the oral consumption of probiotics, namely L. reuteri or a mixture of four probiotic bacteria and prebiotics [30, 31].

Probiotics in Diaorrheal Diseases

There are several etiology for the development of diarrhea, including rotavirus infection in infant, community food and water borne gasteroentritis (norovirus), protozal infection, enteropathozen infection (E. coli) radiation therapy, antibiotic and antimicrobial therapy and also the tubing feeding. The rationale for using probiotics is based on the assumption that they act either by modify the compostion of colonic microbial flora or by modulating the immune response. Probiotics modify the composition of microbial flora by:

1. Compete with pathogens for nutrients and receptors [32].
2. Induce hydrolysis of toxins and receptors [32].
3. Induce production of antimicrobial substances (including peptides of the innate immune system) [32].
4. Induce production of organic acids and modulation of nitric oxide synthesis [32].
5. Regulate intestinal permeability by modulating the epithelial tight junctions [33]. Inhibit selected intracellular mechanisms involved in viral replication (such as MEK, PKA, p38 MAPK) [34].

6. Exert a tropic action on the intestinal mucosa, which leads to brush border enzyme activation, stimulation of glucose absorption and anti-apoptotic effects on the enterocyte [35].

On the other way, probiotics modulate the immune response. Dendrite cells and toll-like receptor molecules are play crucial role in this process. These cells receive signals from structural lipopolysaccharides, glycopeptides and CpG DNA of probiotic strains and transduce them in order to regulate the production of innate immunity peptides that, in turn, exert antimicrobial activity or modulate adaptive immunity [32-34].

Particular probiotics promote specific antibody responses against given pathogens. This is the case of S. boulardii that, apart from producing a 54-kD protease that hydrolyzes the A and B toxins of C. difficile and their intestinal receptors, also stimulates the production of specific IgG and IgA antitoxin A produced by the same pathogen [35].

Antibiotic associated diarrhea

Antibiotic associated diarrhea (AAD) is a common complication of most types of antibiotics, especially for broad-spectrum antibiotics such as clindamycin, beta-lactams and 3rd generation cephalosporins. The non-selective action of antibiotics perturb the normal microbial flora of GIT, which leads overgrowth and multiplication of pathogenic microorganisms i.e, C. difficle, Clostridium perfringens, Staphylococcus aureus, Klebsiella oxytoca, Candida spp and Salmonella spp., ultimately result the production of toxin, leading diarrhea[36]. .

Perturbation of normal microbial flora leads the decrease in the number of bacteria involved in the production of short chain of fatty acids. These fatty acids are important for the nutrition of the enterocyte and for water and electrolyte absorption, and their decrease may result in watery diarrhea. Erythromycin is special case of antimicrobial agents which directly stimulate the motilin and induce the diarrhea.

Recent clinical studies, suggest that oral administration of selected probiotics strain along with antibiotics can be the better option to handle the situation of antimicrobial associated diarrhea. Kotowsha et al. enrolled 269 children who were taking antibiotics for ear or respiratory infections and randomized them to either Saccharomyces boulardii (500 mg/d) or placebo for the duration of the antibiotic treatment. Even though the follow-up time was short (two weeks post-antibiotic), the frequency of diarrhea in the probiotic group was significantly less (3.4%) compared to 17.3% in the placebo group [37].

Cl. difficile associated diarrhea:

The antibiotics that cause C difficile–associated diarrhoea (CDAD) most often are clindamycin, cephalosporins, ampicillin, and amoxicillin. Clostridium difficile has been associated with symptomatic diarrhoea since being identified as the pathogen responsible for

pseudomembranous colitis [38]. Total flora replacement or faecal bacterio therapy has been described as an effective treatment alternative in severe C difficile infections. It is based on transfer of faecal flora from a healthy individual to a severely ill patient. Total flora replacement has also been used to manage severe constipation, irritable bowel syndrome, and inflammatory bowel disease. Homologous faecal enemas have been used in recalcitrant cases of CDAD usually with stool donated by the patient's partner. It is an adjunctive therapy in sporadic clinical use. Administration of donor stool via a naso gastric tube in 18 cases of recurrent CDAD, leading to benefit in15, has been reported. The patients usually receive, after a preceding washout, about 200 to 300 mL of fresh feces dissolved in an equal amount of saline solution. The process is repeated for about 5 to 7 days [39,40].

Billeret al., reported a series involving four children with at least three recurrences of C difficile who were successfully treated with lactobacillus. Use of S boulardii did prevent disease recurrence, but only in those individuals who had more than one C difficile infection sequentially, in the largest, randomised, controlled trial assessing its use in C difficile–associated colitis [41].

Electrolyte absorption

In this context, it has recently been shown that unidentified extracellular proteinaceous compounds secreted by L. acidophilus and L. rhamnosus increased the chloride/hydroxyl exchange activity (Cl_2/ H_2O) in Caco-2 cells through the PI-3K signal transduction pathway. This triggered an increase in Cl_2 absorption, which was also related to an overproduction of the apical anion-exchange transporter SLC26A3 on the cell surface [42]. This increased electrolyte absorption in epithelial cells might be an alternative treatment for people suffering from diarrhea or other intestinal disorders in which electrolyte absorption is perturbed.

Travellers' diarrhoea

Acute diarrhoea occurs in about half of travellers who visit high-risk areas. Although most cases are mild and self-limiting, there is considerable morbidity. Antibiotics are an effective means of prophylaxis but are not recommended for widespread use. Hence there is a need for cost-effective alternative treatments. There have been five blinded, controlled trials studying the efficacy of probiotics in traveller's diarrhea but only one, which assessed S. boulardii, showed significant effects. Saccharomyces boulardii seems to prevent bacterial diarrhoea more effectively, while LGG has been shown to be more effective against viral and idiopathic diarrhea [43].

Infective diarrhea

Rotavirus is the leading cause of infantile diarrhea worldwide and rapid oral rehydration is the primary treatment. Several potential mechanisms have been proposed for how lactobacilli reduce the duration of rotavirus diarrhea viz; competitive blockage of receptor sites

in which lactobacilli bind to receptors, enhanced immune response, signal(s) from lactobacilli that regulates the secretary and motility defenses designed to remove perceived noxious substances and that lactobacilli produce substances that inactivate the viral particles. The probiotics most frequently studied for treating acute diarrhea include LGG and Lactobacillus reuteri [38, 43].

Probiotic in genitourinary tract infection

Unnecessary receive of Broad-spectrum antibiotic (excreted out from urine), disrupt normal flora, and causes symptomatic urinary tract infections such as bacterial vaginosis and candidal vulvovaginitis. The genital tract of premenopausal woman mainly composed of Lactobacilli, especially Lactobacillus crispatus and Lactobacillus iners. These lactobacilli engaged in the production of bacteriocins, lactic acid and hydrogen peroxide, which maintain the pH acidic and keep away the pathogenic colonies from proliferating. Imbalanced microbial flora offer an opportunity for the proliferation of pathogenic microorganism such as E. Coli, Gardnerella vaginalis, Bacteroides sp, beta-Streptococci , Mobiluncus or Falcivibrio sp.and Candida albicans. Restoring the normal flora with lactobacilli may help to treat this genital infection.

Recent trend to treat the UTI includes oral antimicrobial regimens clindamycin, metronidazole or nofloxacin for bacterial vaginitis and fluconazole for candidal vaginitis. Nevertheless, their nonselective antimicrobial action decrease the lactobacilli count, and alter the pH (shift to alkaline), which ultimately associated with recurrence of the infection. Use of effective probiotics could be the best option to manage the above condition and to break the cycle of recurrence.

In a past study conducted by Hilton et al. on 28 women suffering form recurrent VVC.administration of vaginal suppositories cantaining LGG twice daily for seven days, all of the women reposrted an improvement in vaginal symptoms and reduction in vaginal erythma and discharge [44].

The prevention or resolution of bacterial vaginosis is particularly important in women at risk of human immunodeficiency virus (HIV) infection. Studies have shown that women with bacterial vaginosis (no lactobacilli) are at significantly increased risk of HIV [45].Thus, treatment of bacterial vaginosis and promotion of vaginal lactobacilli may reduce a woman's risk of acquiring HIV-1, gonorrhea, and trichomoniasis. A recent publication has shown that a human vaginal probiotic strain (Lactobacillus reuteri RC-14) can express potent functional viral inhibitors which may potentially lower the sexual transmission of HIV [46].

Probiotics in Hypercholesterolemia

There are several mechanism proposed by which probiotics preparations could control blood cholesterol level i.e., by assimilation of cholesterol in the small intestine, and by incorporation of cholesterol into the cell membrane.

In a previous study, Noh et al. found that cholesterol uptake by Lactobacillus acidophilus ATCC 43121 occurred during growth. However, most assimilated cholesterol recovered from the cells was not metabolically degraded. Therefore, the authors suggested that the removal of cholesterol might also due to the ability of L. acidophilus ATCC43121 to incorporate cholesterol into cellular membranes during growth [47].

Another possible mechanism involves the ability of probiotics to ezymatically deconjugate the bile acids by bile salt hydrolase (BSH; chloroglycine hydrolase), enzyme that catalyzes the hydrolysis of glycine and/or taurine-cojugated bile salts mainly occurs in the small and large intestines of mammalian hosts. Previously reported that the removal of cholesterol by L. reuteri CRL 1098 was closely related to the BSH activity of the cells, which hydrolyzed the amide bond of bile salts releasing the corresponding free bile acids. Conjugated bile salts are readily absorbed into the gastrointestinal tract due to higher hydrophilicity, while free bile acids are less soluble and thus less efficiently reabsorbed into the intestines, compared to conjugated bile salts, and thus are more prone to be excreted with the feces. This will increase the need for the synthesis of new bile acids to replace the lost ones. Since cholesterol is the precursor for the de novo synthesis of new bile acids, the use of cholesterol to synthesize new bile would lead to a decreased concentration of cholesterol in blood [48, 49].

Probiotics in Hypertension

Probiotics have potential to regulate blood cholesterol level in hypercholesterolemia. Hypercholesterolemia is one the major cause for hypertension [48]. The elevation of blood pressure found to be greatly induced when total cholesterol level exceeds 6.4 mmol/L. This may increase cardiac output and peripheral vascular resistance that causes an elevated blood pressure. Therefore, lipid metabolism disorders are often the causes of hypertension. A variety of past in vitro experiments and in vivo trials have provided experimental evidence to support the roles of probiotics in lowering serum cholesterol and improving lipid profiles, which subsequently leads to a reduced risk of hypertension.

Further research needed to elucidate the precise molecular mechanism of action of these probiotics in both hypertension and Hypercholesterolemia. This will contribute to the understanding of how probiotics exert these beneficial effects on the human host.

Probiotics in Diabetes

The consumption of probiotics is a new therapeutic strategy in preventing or delaying the onset of diabetes and subsequently reducing the incident of hypertension. High fructose diets induce type II diabetes that is associated with insulin resistance, hyperinsulinemia, hypertriglyceridemia and hypertension. This caused by the mobilization and accumulation of fructose in the liver that increases the rate of lipogenesis and synthesis of triacylglycerol. The catabolism of fructose ultimately induces insulin resistance. In a study conducted by Yadav, Jain and Sinha, it was found that the administration of Dahi (an Indian fermented milk product) containing Lactobacillus acidophilus, L. casei and L. lactis to high fructose-induced diabetic rats for eight weeks decreased the accumulation of glycogen in the liver of rats compared to the control that was not fed the probiotics [50].

Past studies have demonstrated the beneficial effects of co-consumption of probiotics with diabetic drug on controlling diabetes. Gliclazide is an oral anti-diabetic sulfonylurea drug that has beneficial extra pancreatic effects when insulin therapy is insufficient. Al-Salami et al., evaluated the effects of probiotics on the uptake of gliclazide by using diabetic and healthy Wistar rats (n = 10). These rats were fed probiotics (75 mg/kg body weight) for three days, after which a gliclazide suspension (20 mg/kg) was administered. The authors reported that a two-fold probiotics-induced increment($p < 0.01$) in gliclazide uptake was observed in the diabetic rats that resulted in a reduction of blood glucose levels by two-fold via insulin-independent mechanisms. Such findings, point toward the beneficial effects of probiotics for treating diabetes in synergism with other diabetes drug and thereby reduced the incidence of diabetes related hypertension [51].

Probiotic in Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease (IBD) is a collectively term, used for ulcerative colitis (UC) and Crohn's disease (CD). IBD is an abnormal immune response against luminal antigen of commensal bacteria. In case of ulcerative colitis, the composition of microflora is imbalanced associated with increased number of pro-inflammatory bacteria, including Enterobacteriaceae, and Bacteroides fragilis and decreased count of protective bacteria, including lactobacilli and bifidobacteria. Traditionally medication used in IBD includes 5-aminosalicylic acid (5-ASA) and corticosteroids.

Crohn's Disease

In Crohn's disease, commensal E. coli strain stimulates the release of tumor necrosis factor–α (TNF- α) and interleukin-8 (IL-8) by inflamed mucosa. However, some lactobacilli strains including L. casei down regulate the spontaneous releases of TNF- α and the inflammatory response induced by E.coli**.** Therefore, it makes sense either to eliminate some bacteria with antibiotics or to alter the gut flora, in favor of more beneficial bacteria, by the use of probiotics and prebiotics (prebiotics are dietary substances usually non-digested

carbohydrates that stimulate the growth and metabolism of protective commensal enteric bacteria) [52].Proposed mechanisms include:

- Changes in short chain fatty acids (SCFA) production patterns,
- Reduction in pro-inflammatory cytokine secretion, improving Th1/Th2 ratios,
- Eliminating pathogens.
- Enhancement of barrier function

In 1917, it was confirmed that E. coli Nissle is a nonpathogenic E. coli, which colonize the intestine and inhibits the growth of enteropathogenic and other enteric bacteria. It is proposed that this organism, by suppressing enteropathogenic bacteria, may have a long-term effect to suppress remission. In a study by Malchow, 28 patients of active CD were treated with a tapering dose of prednisolone and either placebo or E. coli Nissle. The E. coli Nissle was given in an increasing dosage over 24 days to the final dose of $5x10^{10}$ bacteria per day for a year. The patients are assessed for the remission .There was higher relapse rate in the placebo group (63.6%) as compared to the E. coli group (33.3%) [53].

Fujimori et al, treated 10 patients of CD not responding 5-ASA and prednisolne therapy with a synbiotic therapy, (Probiotic-Bifidobacterium and Lactobacillus and Prebiotic- psyllium), for 12 months. Six patients went into remission, one had a partial response with improvement of the number bowel movements, and three patients were non-responders. There was no significant difference between C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values were observed before and after the therapy [52].

Ulcerative colitis

There is very little clinical trial experience in the treatment of active UC with probiotics. One of the few double blind studies for the patients with active UC, include the combination of probiotics strain, Bifidobacterium longum, with a prebiotic composed of an inulin-oligofructose (growth substrate) on 18 patients. Nine patients assigned to the treatment group and nine to the placebo group. Patients assessed with a clinical activity index, level of gut inflammatory markers and histological sore. The patients were treated with the synbiotic mixture (probiotics-prebiotic) of placebo twice-daily for 4 weeks. At the end of the month, the patients were reassessed. The patients receiving the synbiotic mixture exhibited reduced mucosal inflammatory markers in colonic biopsies. There was an improvement microscopically .There is significant reduction in TNF-α and IL-α levels in mucosal biopsies in patients treated with the active therapy as compared to placebo [54].

Probiotics in Pouchitis

Pouchitis is an idiopathic inflammatory disease of the ileal pouch that occurs in 15%–53% of patients who undergo total abdominal colectomy with ileal pouch-anal anastomosis (IPAA) for UC. Fecal stasis with immunologic reactivity appears to be important in the

pathogenesis of this disease. As a result, this area of study has garnered the most support for the use of and efficacy of probiotics in the IBD [55].

Welters et al. [56] demonstrated that using the dietary fiber Inulin (24 g/day), for the shot period of 3 weeks significantly decreases the pouchitis as measured by endoscopic and histologic score. The results correlated with a significant alteration of fecal pH, fecal butyrate, and secondary bile acid concentration.

In a randomized, double-blind, placebo-controlled study 40 patients were randomized within a week after surgery to receive either VSL#3 (3 g, 9 x 10^{11} bacteria/day) or placebo for 12 months **[57].** Two of 20 (10%) of the patients treated with VSL#3 developed an episode of acute pouchitis compared to 8/20 patients (40%) in the placebo group ($P < 0.01$). Patients treated with VSL#3 and no signs of pouchitis had a median stool frequency of 5 (range, 3–9) at the end of the trial compared to 8 (range, 6 –12) in the placebo group (with no signs of pouchitis; $P < 0.001$).

Probiotics in Cancer

The microbial flora and the immune system of the body play an important role in the modulation of carcinogenesis. Both may be influenced by the probiotics. It has been hypothesized that probiotic might decrease incidence of cancer by:

- Decreasing the exposure of microbial flora to chemical carcinogens
- Detoxifying ingested carcinogens,
- Decreasing the population or metabolic activity of bacteria that may generate carcinogenic compounds
- Producing compounds that inhibit the growth of tumor cells
- Stimulating the immune system to defend better against cancer cell proliferation
- Producing metabolic products (e.g. butyrate) which improve programmed cell death (apoptosis).

It has been thought that the preventions or delay in development of intestinal tumors by lactobacilli is because of its binding to mutagenic compounds in the intestine and suppress the growth of causative bacteria, which convert procarcinogens into carcinogens. The ability of lactobacilli to reduce the risk of cancers has also been based on their ability to modify gut microflora and to decrease B-glucoronidase and other carcinogen levels.

In another study, Reddy et al developed an azoxymethane-induced aberrant crypt foci in colon of rats found that a stimulated growth of bifidobacteria in the colon could lead to the inhibition of colon carcinogenesis. The authors suggested pH-lowering effect of bifidobacteria in the colon, which subsequently inhibited the growth of E. coli and clostridia. A decrease in growth of such pathogenic microorganisms may also produce the modulation of bacterial enzymes such as beta-glucuronidase that can convert pro-carcinogens to proximate

carcinogens. In addition they also suggested that, whole cells of bifidobacteria have also been found to bind with the ultimate carcinogen methylazoxymethanol and mutagen-carcinogen 3-amino-1,4- dimethyl-5H-pyrido[4,3-b]indole, thus physically removing it via feces and subsequently minimizing its absorption into the intestinal lumen [58].

Prebiotics and Synbiotics in cancer

Most of the protective effects of prebiotics on colon cancer have emphasized on the oligofructose-based prebiotics such as fructo oligosaccharides and inulin. In one of the animal trial conducted (trial of 31 weeks) by Femia et al, reported that the protective effect of probiotics on azoxymethane-induced carcinogenesis was less compared to the effects of prebiotics (oligofructose-inulin). The combination of Bifidobacterium and oligofructose synergistically retarded colon carcinogenesis in rats compared to when both given individually [59].

CONCLUSION

Majority of the health complication aroused from the imbalance of the microbial flora of GIT. Further more the rationale use of antimicrobial and chemotherapeutic agents also nonspecifically decrease microbial count of normal flora, may increase the risk of drug resistance and other opportunistic infections. Probiotics therapy is an alternative option to established and restore the microbial flora. More over from the survey of literature, probiotics have potential to treat the various pathological conditions like allergic, cancer, hyperchloestremia, IBD, diarrhea, urogential infections, diabetes, ponchitis and hypertension etc. via positive modulation of several different physiological systems, apart from its conventional benefits for gastrointestinal health. Probiotics generally considered safe but lack of clinical efficacy and safety reports of specific bacterial strain for a particular condition, it is required to establish the therapeutic profile of probiotics preparations.

To fulfill the promises of probiotics, normal host-flora interactions needed to be better understood. It is expected that in the future, new, well-characterized, scientifically proven probiotic strains with specific health benefits will be developed. In addition, further well-designed clinical trials should provide health care professionals with sufficient evidence for the rational use of probiotics.

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