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Review Article

PROBIOTICS IN DIARRHEA: MYTHS AND FACTS

ABSAR A. QURESHI*, SHAISTA OMER, K. ESWAR KUMAR, N.S.BHAJIPALE

Department of Pharmacology, S.G.S.P.S.Institute of Pharmacy, Hingna Road, Kaulkhed, Akola-444004, Department of Pharmacology, Soniya College of Pharmacy, Dharwad-580002, Department of Pharmacology, University College of Pharmaceutical Sciences, Andhra University, Vishakhapatnum-530003, S.G.S.P.S.Institute of Pharmacy, Hingna Road, Kaulkhed, Akola-444004.
Email: absaraqureshi@yahoo.com

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ABSTRACT

Diarrhea is a common health problem worldwide. Antibiotics, nosocomial infections and microorganism are the common factors responsible for diarrhea. Moreover, imbalance in intestinal microbial flora also leads to diarrhea. Probiotics is a living microorganism administered to promote the health of the host by treating or preventing infections due to strains of pathogens. Probiotics are effective against diarrhea and act by various mechanisms like colonization resistance, production of antimicrobial substances, competitive inhibition for bacterial adhesion sites, anti-secretary effect, and inhibition of toxin binding, enhancement of immune system and tropic effects of intestinal mucosa. Various randomized double blind studies indicate the effects of bacterial and yeast probiotics against various types of diarrhea. Between both types of probiotics yeast probiotics i.e. *Saccharomyces boulardii* is very useful against different types of diarrhea.

Keywords: Diarrhea; probiotics; *Saccharomyces boulardii*

INTRODUCTION

Gastrointestinal diseases are often a consequence of a myriad of factors, which disturb the bowel's complex ecosystem. Diarrhea is associated with an increased frequency of bowel movements with the production of soft or watery stool. It may be defined as the passage of more than 300 ml of liquid faeces in 24 hours. This results in fluid and electrolyte loss that may lead ultimately to death particularly in young children[1].

Antibiotics are the most common culprit of acute diarrhea due to loss of "colonization resistance" or the protective role of normal intestinal flora against pathogenic organism[2]. A great variety of antibiotics have been implicated, but the most frequently associated with diarrhea are penicillins (especially ampicilin or amoxicillin), cephalosporins and clindamycin[3-6]. Over one-third of antibiotic associated diarrhea is associated with an infection by an anaerobic bacterium, Clostridium difficile, which also cause nosocomal (hospital acquired) outbreak[7-9]. In addition, medications and in-hospital procedures have been associated with a higher risk of diarrhea in nosocomal outbreaks[10, 11]. Host factors such as advanced age, gender and severe underlying disease conditions have been implicated in higher risk of acquiring nosocomial diarrhea[10, 12].

Other etiologies of diarrhea are due to infections not associated with antibiotic predisposition (e.g. Toxigenic, *E.coli* and *Vibrio cholerae*, or infection with *Entomoeba histolytica*, *Giardia lambia* or viruses). In many instances of acute diarrhea in children, hospitalized patients or HIV-infected patients, the etiological agent has not been determined. The traditional treatment for acute diarrhea often depends on weather a known etiological agent can be identified, on the severity of symptoms and the source of the infection (community or nosocomial). Electrolyte replenishment and cessation of the inciting agent (antibiotic or medication) are often all that is required for the treatment of milder forms of diarrhea. Specific therapy may be prescribed if a specific etiological agent can be detected.

Unfortunately, these steps are not always sufficient and the diarrhea may continue and became chronic, symptoms may increase in severity and spectrum or toxic mega colon or death may ensue[3, 13].

In an effort to prevent or treat these difficult cases of diarrhea and to also re-establish the normal homeostasis of the colonic ecosystem, innovative approaches have been tried using living, biotherapeutic agents.

Physiological roles of the intestinal microflora

There are about 400 different species of flora in the intestine of each human being, and about 10 fold more bacteria than the number of human cells[14, 15]. The dominant flora is characterized by the presence of more than 10^8 bacteria per gram of faeces, and is anaerobic (Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus, etc). The establishment of the neonatal, infant and adult colonic microflora is a gradual, sequential process[16, 17]. The intestinal micro flora has many following functions as reviewed by Midtvedt[18].

- Microflora offers protection against intestinal colonization with pathogenic microorganism and regulates intestinal transit.
- The intestinal wall is enlarged in the presence of intestinal microbes.
- Migrating motor complexes, production and sensitivity to peptides are dependent on the microflora.
- Deconjugation of bile acids and promotion of the enterohepatic circulation, degradation and digestion of some undigested carbohydrates, improvement of lactase tolerance, production of vitamins and growth factors for host intestinal cells.
- Intestinal flora maturates and stimulates the gut immune system.

Situations of imbalance in intestinal microflora and digestive disorders

Various digestive disorders in adults and / or children may be associated with imbalance of the intestinal flora like certain types of acute and chronic diarrhea, irritable bowl syndrome, chronic inflammatory diseases of the intestines[19]. Antibiotic associated diarrhea (AAD) is the most typical example of a pathological situation related to imbalance of the intestinal flora, with incidence rate of 11%[20]. The pathophysiology of AAD may involve the implantation of an enteropathogenic organism, as the result of damage to barrier flora by the antibiotic.[21]

PROBIOTICS

The Russian Metchnikov, who was awarded the Nobel Prize for medicine in 1908, for demonstrating that some bacteria could stimulate the growth of Vibrio cholera, while others did inhibit its growth, first coined the theoretical concept of "probiotics" or "biotherapy"[22].

A "Probiotic" or "biotherapeutic agent" is a living microorganism administered to promote the health of the host by treating or preventing infection due to strains of pathogens[23-25]. There are increasing experimental and clinical data to support probiotics use in the prevention and treatment of many gastrointestinal disorders, including inflammatory bowel disease, infectious and antibiotic related diarrhea and post surgical disorders[26, 27].

In an effort to reduce the use of antibiotics in the face of increasing development of antimicrobial resistant bacteria, the WHO has advocated, where possible, a policy of microbial interference therapy; the use of non-pathogens to eliminate pathogens[28-31].

Classification of probiotics

Globally, biotherapeutic agents can be divided into following two groups (Table 1).

Table 1: Classification of Probiotics

Bacterial Probiotics	Yeast Probiotics
Lactobacillus acidophilus *Lactobacillus casei GG* *Bifidobacterium bifidum* used with *Streptococcus thermocephilus* *Enterococcus faecium SF68*.	*Saccharomyces boulardii*.

Mechanism of action of probiotics

Here we cover mechanism of action of bacterial probiotics and yeast probiotics simultaneously.

Colonization resistance

Colonization resistance is the property of the normal colonic flora for protecting against colonization by pathogens, and is due to a complex interaction of various strains of colonic bacteria, which make up the microflora. A probiotics needs to inhibit the proliferation of pathogens during a period when the normal colonic microflora is disturbed[23].

Production of antimicrobial substances

Lactobacillus casei GS has been shown to produce inhibitory substances in vitro towards a broad spectrum of gram positive and gram-negative pathogens[32]. It also produces in vitro hydrogen peroxide, which is bactericidal[33]. Yogurt, which contains S. thermophilus and L. bulgarius, has a bactericidal activity against *Clostridium difficile* in vitro[34, 35].

Competitive inhibition for bacterial adhesion sites

A Lactobacillus strain was shown to competitively inhibit adhesion of enteropathogenic *E.coli* to pig ileum and interfere with bacterial attachment to the mucosal layer of ileal conducts[36].

L. acidophilus strain can attach in vitro to cells resembling to enterocytes, which other strain of *L. acidophilus* do not[37, 38]. Exposure of *Entamoeba histolytica* trophozoites to *S. boulardii*, its membrane or yeast culture supernatants decreased the number of Arophozoites able to attach to erythrocytes in vitro[39] *S. boulardii* also inhibited in vivo the proliferation of *C. krusei* and *C. pseudotropicalis* but had no inhibitory action on *C. tropicalis* by the same mechanism[40].

Anti-secretory effect induced by toxins

Vibrio cholerae produces a toxin, which activates adenylate cyclase of the enterocyte and stimulates cAMP production resulting in a major secretory diarrhea. *S. boulardii* inhibit cholera induced secretion in rabbit jejunum[41].

Inhibition of toxins binding to intestinal receptors

Clostridium difficile is the most frequent cause of nosocomial diarrhea in adults and the pathogen causing persistent and protracted enterocalopathies[42,43] and pseudo- membranous enterocolities in children[44] as well as adults[45] Carthier et al found that gnotobiotic mice, who generally die rapidly after a *C. difficile* challenge, were protected after a single dose of *S. boulardii*[47]. Several studies have shown that *S. boulardii* inhibited the formation of histological lesions in the cecum due to the toxins of *C. difficile* in mice and hamster[47-49].

Enhancement of the immune defense system

Oral ingestion of *S. boulardii* causes significant increase in the production of secretory IgA and of the receptor for polymeric immunoglobulins in growing rat small intestine. In a study the decreased proliferation of systemically administered *Candida albicans* by treatment with *S. boulardii* presumably proceeds by same similar mode of immune stimulation[50-52].

Trophic effect on intestinal mucosa

Very high doses of spermine and spermidine given to young test animals cause a significant increase in the length and weight of the intestinal system, and accelerated adult response of the enzymes of the microvilli (lactase, sucrase, maltase and aminopeptidase) and an increase in the secretory component of the immune globulins in both the villi and the crypt cells[53,54]. A significant increase in secretory IgA and the secretory component in rats treated with *S. boulardii*[52] and an increase in disaccharidase activity (lactase, sucrase, maltase) in the mucosa of the small intestine has been detected in test subject[55,56].

Randomized double blind studies of probiotics

Here in this review we have covered randomized double blind placebo controlled clinical studies of both bacterial and yeast probiotics separately against diarrhea caused by common etiologies (Table 2 and 3).

Side effects of bacterial probiotics

- **Malabsorption and metabolic acidosis**

Disturbances of balance between gram-positive and gram-negative bacteria in the natural colonic flora might be inducing by the administration of gram-positive bacteria such as lactobacilli and bifido bacteria that have a stronger growth than gram-negative bacteria. This imbalance might result in metabolic D-lactate acidosis, as a consequence of the bacterial carbohydrate metabolism[75].

- **Bacteraemia**

Lactobacillus bacteria are not pathogenic although endocarditis, meningitis pneumonia and sepsis have been reported[76, 77].

Arthritis and immunosuppressive effect

Injection of the cell membranes of streptococci and Lactobacilli can cause chronic polyarthritis. Bacterial components may possibly be transported to the joints, where they trigger off a local reaction[78, 79].

Side effects of yeast probiotics

The safety of *S. boulardii* has been investigated in animal models and in randomized double blind studies. In mice given 5% *S. boulardii* for 70 days in their drinking water, no translocation from the gastrointestinal tract was observed. S. boulardii could not be detected in the organs (liver, kidney, lungs and heart) or in the mesenteric lymph glands[80]. In an immune depressed animal model (prednisolone and antibiotic decontamination) *S. boulardii* could

only be detected at very low concentration in the mesenteric lymph gland[81]. *S. boulardii* was given to more than 40 AIDS patients without any serious side effects being reported[74, 82, 83]. Within a period of 10 years, during which millions of *S. boulardii* treatment has been prescribed, few cases of fungaemia with *S. boulardii* have been reported[84].

Table 2: Randomized double blind placebo controlled studies of Bacterial probiotics

Sr. no	Type of bacterial probiotics	Objective	n⁰	Inhibition by probiotics (%)	Inhibition by placebo (%)	p	Ref.
01	(Living)	A*	48	70	68	N.S.	57
	L.bulgaricus-	B*	50	35	29	N.S.	58
	L.acidophilus	C*	353	01	11	N.S.	59
02	(Living) *L. acidophilus*	B*	319	53	47	N.S.	60
03	(Unspecified) *L. acidophilus*	B*	202	25.7	23.8	N.S.	61
04	(Heat Killed) *L. acidophilus*	D*	712	5.4	25.50	N.S	62
05	*Lactobacillus G.G.*	B*	820	41	46.50	N.S	63
06	*L. fumentum*	B*	181	23.8	23.8	N.S	64
07	*Streptococcus*	E* E*	45	8.7	27.2	N.S	65
	faecium SF 68	F* F*	39	24 Hrs.	24 Hrs.	N.S.	66

Objective**:** topic of the study; A) Prevention of diarrhea in volunteers infected with ETEC; B) Prevention of traveler's diarrhea; C) Prevention of diarrhea associated with enteral feeding; D) Acute diarrhea in children; E) Prevention of AAD; F) Mean duration of diarrhea in adult with acute diarrhea; G) Prevention of diarrhea in hospitalized children.

n°: no of patients ; p: level of significance; AAD: antibiotic associated diarrhea; *ETC*: enterotoxic E. coli

*Parameter that was statistically evaluated

Table 3: Randomized double blind placebo controlled studies with *S. boulardii*

Objective	n°	*S. boulardii*	Placebo	p	Ref.
Prevention of AAD*	388	4.5%	17.5%	p<0.001	67
Prevention of enteral feeding diarrhea*	40	8.7%	16.9%	p<0.001	68
Prevention of enteral feeding diarrhea*	20	1.5%	9.1%	p<0.001	69
Patients with severe burns	1231	31.8%	42.6%	p<0.002	60
C. difficile colitis*	124	26.3%	44.8%	p<0.05	70
Children's acute diarrhea*	130	15%	60 %	p<0.01	71
Adults with acute diarrhea*	92	3%	12 %	p<0.05	72
Children's chronic diarrhea*	40	30%	90 %	p<0.001	73
AIDS patients-chronic diarrhea*	35	39%	88 %	p<0.002	74

Objective: topic of the study; n°: no of patients; p: level of significance

AAD: antibiotic associated diarrhea; ETC: enterotoxic *E. coli*

*Parameter that was statistically evaluated

Safety of probiotics

The safety of viable microorganism is difficult to establish using the current assessment methods; theoretically, almost any microorganism may, under certain circumstances, either cause an infection or alter its virulence due to internal or external factors. The balance between intestinal microbes, the intestinal barrier and the whole body forms a cohabitate in which the human system accepts the microbial interference and ecology in its current form. No evidence of opportunistic infection or other ill effects by probiotics has been observed nor have harmful effects been observed in controlled clinical studies with lactobacilli and bifidobacteria[84, 85].

Also the nonpathogenic S. boulardii yeast has been shown to be safe in animal models and although the yeast has been available for over 40 years and used throughout the world, side effects are uncommon[86]. Nonetheless a few cases of fungemia have been reported although only 13 well documented cases of S. boulardii infection have been described in the literature[87, 88]. In one study constipation and increased thirst were reported to occur[70].

In conclusion, the reported risks of biotherapeutic agents are extremely rare. Cases reported in the literature are limited to sporadic cases of transient bacteremia or fungemia[87].

CONCLUSION

In health, the gastrointestinal tract is not only an organ of digestion and absorption which is metabolically active and has specific nutrient requirements, but it has an additional function as a major barrier, protecting the body from harmful intraluminal pathogens and large antigenic molecules.

Probiotics offer a large number of theoretical advantages in the treatment of diarrhea. They offer a possible solution to the problem of the ever-increasing resistance of bacteria to antibiotics and have the advantage that they work by multiple pathophysiologic mechanisms, as a result of which the development of resistance is unlikely without altering the colonization of resistance of the intestinal flora.

These probiotics can competitively prevent pathological bacterial colonization strengthen epithelial tight junctions and stimulate subsets of T-helper cell[89- 91]

S.boulardii is the only probiotics with convincing and reproducible double blind studies[92] and statistically significant efficiency in the prevention and treatment of diarrhea. *S. boulardii* and loperamide are the only antidiarrhoeal on the positive list [93]; the WHO regards *S. boulardii* as a possible treatment for recurrent *C. difficile* colitis[94].

Although some in vitro studies provided very interesting results, their clinical relevance in vivo would often not be confirmed, or need superphysiological concentrations of probiotics. For this reasons only the double blind placebo controlled studies are evaluated (Table No. 2 and 3).

Except S. boulardii, all other probiotics still need to demonstrate their effectiveness in well-designed randomized double blind studies before they can be recommended on a large scale[92]. Not a single bacterial probiotics appears clinically effective in the prevention and treatment of diarrhea in randomized double blind studies (Table 3).

If a bacterial probiotics were to be used on a large scale in the future, one would have to be certain that it was not implicated in antibiotic resistance transfer, especially if it was intrinsically resistant to particular antibiotics. Investigation must also be made to find out whether lactic acid producing bacteria are not contraindicated in indication where malabsorption may be develop which may lead to metabolic acidosis. Research must be carried out on the immunologically toxic behavior of bacterial peptidoglycan derived from the cell wall of gram positive bacteria and the possible consequences of use in diarrhea with increased permeability (e.g. salmonellosis).

The risk of probiotics getting into the blood stream is a theoretical side effect for all probiotics. *S. boulardii*, which has been widely used for years, appears to be relatively safe : only 7 cases in a period of 10 years, during which millions of treatments per year have been prescribed, have been published in well-documented clinical cases. S. boulardii gives promising results in AIDS patients without any serious side effects being reported. This extensive experience of use is lacking for bacterial probiotics and very few probiotics are available which describes the risk of translocation to the blood, although a considerable risk was found in immunodepressed (cancer) patients during *Bacillus cerus* therapy, and caution is urged when using lactobacilli, more particularly L. rhamnosus, in immunodepressed patients.

However the use of probiotics must be carefully considered when probiotics are used in patients at high risk for opportunistic infections or when the gastrointestinal tract is badly damaged. In conclusion, the health claims of bacterial or yeast probiotics require rigorous assessment and their underlying mechanism of action need to be thoroughly understood before any final statements on the clinical value of probiotics against diarrhea. Moreover there is need to develop suitable screening and selection methods for new potential strains with properties similar to or superior to the present successful probiotics against diarrhea.

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