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Antibacterial potentials of probiotics; an explorable approach in therapeutic microbiology?

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ABSTRACT

Over the years, probiotics have been shown to have antibacterial potentials through various studies being carried out and thus can be introduced in the course of treatment for bacterial infections. Probiotics act through competitive inhibition, direct antagonism of pathogens and production of antimicrobial factors, they deprive invaders of nutrients, secrete acids that pathogens cannot tolerate and modulate the immune system. Patho-biotechnology has contributed to probiotic application by adopting processes that improve their physiological stress tolerance and increase the resistance of the probiotic strains to industrial processing so as to ensure gastrointestinal transit in numbers adequate enough to elicit a defined benefit to the host. Given the potential antibacterial properties of probiotics, coupled with the fact that in comparison to conventional therapeutics they are relatively simple and inexpensive to produce, transport and store, they may herald a new era in clinical microbiology, especially for the developing world.

INTRODUCTION

Bacteria exist in abundance; they fill the world, human bodies, the gut, in and on the skin, and in other orifices. Friendly bacteria exist for proper development of the immune system, for protection from pathogenic bacteria, and for the digestion and absorption of food and nutrients. Each person's mix of bacteria varies. Interactions between a person and the bacteria in his body, and among the bacteria themselves, are essential for the person's health. The bacteria- balancing in the human system can be altered by antibiotics and pathogens; there is increased usage of probiotic to offset the action of these alteration agents and research is ongoing to determine the halting or suppression of the agents.

Probiotics are defined by the World Health Organization/ Food and Agricultural Organization as live microorganisms (in most cases, bacteria) which when administered in adequate amounts confer a health benefit on the host; they are similar to beneficial microorganisms found in the human gut. They usually exert their effects by positively influencing normal microbe-microbe and host-microbe interactions. They can be used as complementary and alternative medicine (CAM) [1].

TYPES OF PROBIOTICS

These include the resident bacterial flora, the transient flora and *Saccharomyces boulardii*. The resident flora include Lactobacilli, Bifidobacteria while the transient bacteria include *Bacillus laterosporus, Bacillus subtilis* and *Streptococcus thermophilus*. Lactobacilli are important group of probiotic bacteria that inhibit undesirable microflora in the human gut and create a healthy equilibrium between beneficial and potentially intestinal pathogens. The probiotic *Lactobacillus species* include *L. acidophilus, L. fermentum, L. paracasei, L. brevis, L. gasseri, L. plantarum, L. bulgaricus, L. helveticus, L. reuteri, L. casei, L. jensenii, L. rhamnosus, L. crispatus, L. johnsonii,* and *L. salivarius* [2].

Bifidobacteria produce high degrees of essential by-products in the intestines, which act as barrier to the growth of dangerous pathogenic microbes that cause infection. The probiotic strains are *B. breve, B. longum, B. infantis, B. bifidum, B. lactis, B. thermophilum, B. animalis* and *B. adolescentis* [3].

Bacillus laterosporus is one of the transient friendly micro organisms found in the human gastrointestinal tract. It has been demonstrated in clinical studies to provide unique relief from disease symptoms particularly those associated with suppressed immune system function including bacterial infections, its effectiveness against immune related illnesses is its strong antibiotic qualities [4].

Bacillus subtilis is one of the most important immune system stimulators of all the transient bacteria. It is remarkable for its ability to activate the body's immune defence, as well as its ability to stimulate the proliferation of crucial lymphocytes (5).

Streptococcus thermophilus is a transient micro organism that produces a number of antibiotics- like substances as part of its metabolic process. It increases proliferation of lymphocytes, stimulates B-lymphocyte and macrophage response, and

stimulates the body's immune response through Peyer's patches and intestinal lymph nodes. This aids the body in its ongoing fight against pathogenic bacteria [6].

Enterococcus faecium is found in a number of probiotic products. Various animal model and human experiments have shown its probiotic property [7, 8]. The setback about this bacterium is that *E. faecium* has evolved from a relatively nonpathogenic commensal bacterium to the third most common cause of hospital-acquired infections and now accounts for over 10% of enterococcal clinical isolates, and it has developed extensive resistance to antibiotics, which it is capable of transferring to other bacteria [9].

ANTIBACTERIAL EFFECT OF PROBIOTICS

Scientific studies have revealed that probiotics offer remarkable potential for the prevention and management of various infective disorders as they are able to specifically target bacterial pathogens; there has thus been a resurgence of interest about the strain specific benefits of probiotics and clinical research is quickly accumulating to support the evidence for their use. Over the years, the effects of probiotics on various bacterial infections have been studied.

Probiotics in diarrhoeal infections

Probiotics act against intestinal pathogens and possible mechanisms include the synthesis of antimicrobial substances, competitive inhibition of adhesion of pathogens, modification of toxin and non toxin receptors and stimulation of non specific and specific immune responses to pathogens. One of the evidences for probiotic efficacy is the prevention and treatment of enteric infections. These include acute diarrhoea, chronic diarrhoea which usually arise secondary to malnutrition and immunodeficiency, infectious diarrhoea, irritable bowel syndrome, inflammatory bowel disease and traveller's diarrhoea [10, 11].

Oral probiotic therapy supplementing oral rehydration treatment has in the recent years been suggested to have a substantial therapeutic potential for reducing the severity and duration of diarrhoea. Probiotic bacteria have been shown to significantly reduce both the frequency and duration of diarrhoea associated with infections linked to malnutrition [12]. The beneficial effects of probiotics are strain dependent, dose dependent, greater for doses of more than 1010 colony forming unit (c.f.u.), and more evident when treatment with probiotics is initiated early in the course of the disease [13].

It was demonstrated that consumption of *L. reuteri* shortened the course of acute diarrhoea in infants from two and half to one and half days [14]. Similar effects were observed with *L. rhamnosus* which also significantly reduced the duration of hospitalization [15]. *Lactobacillus* GG improves colonisation resistance and protects the intestine from harmful bacteria; a study [16] showed that *Salmonella* levels were considerably lower in the intestines of mice that received *Lactobacillus* GG than in the placebo group, and the life spans of *Salmonella* infected ex-germ-free mice were considerably extended by *Lactobacillus* GG.

Probiotic cocktails which are combinations of two or more strains with potentially different mechanisms of antimicrobial action have produced better results than individual probiotics. Lower levels of *Salmonella* infection, reduced incidence, severity and duration of diarrhoea were observed in a porcine model of animals treated with a cocktail using a mixture of two strains of *L. murinus* and one strain each of *L. salivarius, L. pentosus* and *Pediococcus pentosaceous* (LIVE5) relative to the controls that were administered skim milk [17].

Use of probiotics in neonates at risk for necrotizing enterocolitis (NEC)

Very low birth weight (VLBW) infants are prone to necrotizing enterocolitis (NEC) and they are predominantly colonized by the pathogenic bacteria as opposed to healthy, term infants in whom Bifidobacteria predominate as stated by Dai and Walker [3]. Since microbial invasion of the gut wall may be a contributing cause of NEC, altering microbial flora by enteral feeding of probiotics might be beneficial. Earlier [18] and recent studies [19] have shown positive effects. Mechanisms by which probiotics may protect high-risk infants from developing NEC include increasing the barrier to translocation of bacteria and bacterial products across mucosa, competitively excluding potential pathogens, modifying host response to microbial products and enhancing enteral nutrition that inhibits the growth of pathogens such as *Klebsiella pneumoniae* and *Escherichia coli* [20].

Antibiotic-associated diarrhoea (AAD)

Probiotics replenish normal microbiotic populations in an individual after a period of antibiotic therapy. Probiotics have been used to prevent antibiotic associated diarrhoea, which results from an imbalance in the colonic microbiota caused by antibiotic therapy. Lactobacilli, Bifidobacteria and *Saccharomyces boulardii* have been reported to be potentially beneficial [21]. *Lactobacillus rhamnosus* GG has been reported to reduce the incidence of diarrhea in patients receiving rabeprazole, clarithromycin and tinidazole (triple therapy) for *Helicobacter pylori* eradication [22]. Efficacy of probiotic AAD prevention is dependent on both the probiotic strain used and the dosage. Up to a 50% reduction of AAD occurrence has been found, and no side-effects have been reported in any of these studies [23].

Helicobacter pylori gastritis

A study reported that a mixture of probiotic organisms (including Lactobacillus bulgarius, Lactobacillus acidophilus and Bifidobacterium lactis) was associated with a significant reduction in Helicobacter pylori infection over a period of 6 weeks in adults while in asymptomatic children, Similarly probiotic formulations (two Lactobacillus species) administered over a 4-week period, significantly reduced Helicobacter pylori activity (24).

Lactobacillus gasseri inhibited both the in vitro growth of clarithromycin-resistant H. *pylori* and the release of interleukin-8 from epithelial cells [25]. In addition, Johnson-Henry *et al.*, [26] noted that a mixture of Lactobacillus strains reduced gastric inflammation and bacterial colonization in *Helicobacter pylori*-infected animals.

Urogenital tract infections

Probiotics are also used to prevent and treat infections of the urinary tract. A case-control study reported that women who frequently consume fermented milk products containing probiotic bacteria, such as *L. acidophilus* or *Lactobacillus GG,* experience fewer urinary tract infections, and there is a significant reduction in the recurrence of urinary tract infections (UTI) following the regular urogenital use of probiotic capsules [27].

A prospective clinical pilot study confirmed the safety and effectiveness of *Lactobacillus* vaginal suppositories against the recurrence of bacterial UTI [28]; it showed that vaginal suppositories with *L. crispatus* GAI 98332 can reduce the recurrence of UTI significantly without any adverse complication during treatment. The route of delivery of probiotic lactobacilli is via direct instillation into the vagina. For example, the weekly application of *L. rhamnosus* GR-1 and *L. fermentum* B-54 was shown to reduce UTI recurrences from an average of 6 to 1.6 per year [29].

In addition, several in vitro studies have revealed probiotics' potential in relieving bacterial vaginosis (BV). In vitro studies have shown that *Lactobacillus* strains can disrupt BV biofilms and inhibit the growth of urogenital pathogens [30]. Vaginal application of probiotics can treat symptomatic bacterial vaginosis [31]. Studies demonstrated that oral probiotic *L. rhamnosus* GR-1 and *L. reuteri* RC-14 treatment augmented the efficacious treatment of BV with metronidazole [32, 33]. The mode of action involves anti-adhesion factors, by-products such as hydrogen peroxide and bacteriocins lethal to pathogens, and immune modulation or signaling effects [34].

Oral infections

Studies have shown that probiotics are effective against bacterial oral infections including caries and periodontal disease. They function in various ways: probiotics can create a biofilm in oral cavity, acting as a protective lining for oral tissues against oral diseases, keeping bacterial pathogens off oral tissues by filling a space pathogens would invade in the absence of the biofilm and by binding in oral cavity, competing with cariogenic bacteria and periodontal pathogens growth for adhesion sites and substrates available through involvement in metabolism of substrates [35, 36]. Probiotics also contribute to prevention of dental caries through the production of organic acids, hydrogen peroxide and bacteriocins [37].

Several studies and clinical trials have demonstrated consumption of products containing probiotics could reduce caries and caries-associated microbes and also the number of *Streptococcus mutans* in saliva [38-40]. In a double-blind, placebo controlled experiment in which 594 children aged 16 years old were given either normal milk or milk containing *L. rhamnosus* GG for 7 months, Nase *et al*., [38], observed that there were significantly fewer cavities in the children receiving the probiotic compared with those receiving normal milk. Krasse *et al*., [41] also demonstrated a significantly reduced gingival index and bacterial plaque amount in patients treated with *L. reuteri* and concluded that this probiotic was effective to reduce gingivitis and bacterial plaque deposition in patients with moderate-to-severe gingivitis.

Strains of oral Lactobacilli have been isolated that are inhibitory against *S. mutans*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *Porphyromonas gingivalis* [42]. In another study, patients with various periodontal diseases, gingivitis, and periodontitis were locally treated with a culture supernatant of probiotic strains including *L. acidophilus*, *L. reuteri* strains, *L. brevis* (CD2), *L. casei Shirota*, *L. salivarius* WB21, and *B. subtilis*. Significant recovery was reported for almost every patient. *L. reuteri* and *L. brevis* have improved gingival health, as measured by decreased gum bleeding [43]; in a like manner, *B. subtilis* reduced the number of periodontal pathogens[44].

The major delivery vehicle of oral probiotic in the management of oral infections is chewing gum; this has probiotic bacteria which consisting of particular strains of *Lactobacillus* that bind to the cariogenic bacteria, clumping them together, thus making them incapable of sticking to the teeth and causing decay. It facilitates rapid drug absorption through the oral mucosa to achieve fast onset of action and bioavailability [35].

Respiratory Tract Infection

Clinical trials have demonstrated that probiotics may decrease the incidence of respiratory tract infections [46]. Leyer, [47] enrolled 109 one-month-old infants and randomly assigned them to receive either twice daily doses of the probiotics *Bifidobacterium animalis* subsp. *lactis* BB12 or a placebo until they reached age eight months. He discovered that infants who had received probiotics experienced a significant reduction in respiratory infections when compared with those in the placebo group.

Otitis media

Intranasal application to infants with recurrent otitis media of a mix of α -streptococcal bacteria shown in vitro to suppress growth of non-typable *Haemophilus influenzae* and *Streptococcus pneumoniae*, significantly reduced the incidence of subsequent episodes of otitis media [48].

PROPERTIES OF AN EFFECTIVE PROBIOTIC

An effective probiotic should: exert a beneficial effect on the host, be nonpathogenic, nontoxic, contain a large number of viable cells, be capable of surviving and metabolising in the gut, remain viable during storage and use, be beneficial for host health, easy to process and cost effective, have good sensory properties and be isolated from the same species as its intended host. Dairy products are mainly used as carriers for probiotics after their extraction (49).

Highly adapted pathogenic stress survival and host evasion strategies are exploited for the development of improved probiotic cultures increase the resistance of the probiotic strains to industrial processing such as drying or heating because a probiotic strain unless protected by a capsule should be intrinsically resistant to low pH, bile and pancreatic enzymes to ensure gastro-intestinal transit in numbers adequate enough to elicit a defined benefit to the host [50, 51].

MODE OF ACTION OF PROBIOTICS

Based on a report by Oelschlaeger, [52], the effects of probiotics may be classified in three modes of action:

(*i*) Probiotics might be able to modulate the host's defences including the innate and acquired immune system. This mode of action is important for the prevention and therapy of infectious diseases and for the treatment of chronic inflammation of the digestive tract or its parts.

(*ii*) Probiotics can also have a direct effect on other microorganisms, commensal or pathogenic ones. This helps in the prevention and therapy of infections and restoration of the microbial equilibrium in the gut.

(*iii*) Furthermore, probiotic effects may be based on actions affecting microbial products like toxins and host products, *e.g.* bile salts and food ingredients. Such actions may result in inactivation of toxins and detoxification of host and food components in the gut.

The kind of effect a certain probiotic produces depends on its metabolic properties; the molecules presented at its surface or on the components secreted, including fundamental parts of the bacterial cell such as DNA or peptidoglycan are all of importance for its probiotic effectiveness. The individual combination of such properties in a certain probiotic strain determines a specific probiotic action and as a consequence its effective application for the prevention and treatment of a certain infection.

In acute infections probiotics boost the protection afforded by commensal flora through competitive interactions, direct antagonism of pathogens, and production of antimicrobial factors, probiotic bacteria produce a variety of substances that are inhibitory to both gram-positive and gram-negative bacteria including organic acids, hydrogen peroxide, bacteriocins and bacteriocin-like inhibitory substances as lactic acid [10], while in chronic infections and immuno-suppression, probiotic action is by microbe-host signaling [52]. Probiotics promote health through activation of the mucosal immune apparatus and there is evidence to suggest that they may improve immune function by increasing the number of IgA-producing plasma cells, increasing or improving phagocytosis as well as increasing the proportion of T lymphocytes and Natural Killer Cells (NKCs) [53].

SIDE EFFECTS OF PROBIOTICS

Some side effects of probiotics are linked to digestive problems. Gas, bloating and stomach cramps are common effects of probiotics, especially in people who are eating probiotics foods alongside with taking probiotics supplements. This results from the destruction of the pathogenic bacteria by probiotics the gut results in gas, bloating and for a few weeks [54]. Some studies indicate that probiotic products like yogurts could be a cause of obesity [55].

In patients on immunosuppressants, impaired immune systems, and those who have a compromised intestinal barrier or underlying health problems, probiotics could over-stimulate the immune system, causing unhealthy metabolic activities, or gene transfer leading to probiotic-caused infections like *Lactobacillus* septicaemia [56], also there have been a few reports of probiotics causing severe fungal infections in such patients [57].

Possibly the biggest potential danger of probiotics is the risk of transferring antibiotic resistance from probiotics to more deadly microorganisms [58]. This is why scientists have strict rules on which bacteria can qualify as probiotic.

Other researchers concluded that probiotic supplements are generally considered safe for use by healthy people: in 143 studies that included a total of over 7,500 participants, no serious adverse effects of probiotics have been noted [59].

CONCLUSION

Probiotic therapy, in combination with conventional therapies helps to fight existing diseases, and protect against future infections since probiotics have the potential to fight infection, and to modulate the immune system. On the part of delivery vehicle, fermented dairy products are a convenient, culturally acceptable and safe method to increase probiotic intake.

In the developed countries, probiotic knowledge abound and its application is vast but in developing world like in Africa, probiotic research and use is very limited and there is need for more research in probiotics. Support for basic and clinical studies examining local customs of using fermented foods to determine which products deliver the health benefits of probiotics should be encouraged and mechanisms for promoting clinically proven probiotics must be developed.

Probiotic research has really gained scientific credence in the last decade, but more scientific knowledge is needed about probiotics, including production of probiotics that harbor high numbers of viable organism at the time of consumption, their safety and appropriate use to get the maximum effect. Three levels of study are necessary to ascertain the safety and efficiency of probiotics: *in vitro* studies, animal studies, and ultimately, clinical trials. Controlled clinical trials have been used in safety assessments of probiotics but more research still need to be done in this aspect, and history of safe use over a period of time studied, especially regarding the probiotics that are not considered safe in application, like *Enterococcus faecium*.

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