MEDICAL PRACTICE

Probiotics: current trends in the treatment of diarrhoea

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In recent years, research into and public interest in probiotics and probiotic foods have risen. Lactobacilli and bifidobacterium are the most commonly used probiotics while yoghurt and kefir are popular foods containing probiotics. Probiotics have been used to manage diarrhoea. Many things cause diarrhoea, including bacterial, viral and protozoal infections, radiation and antibiotic therapy. Different studies have found that probiotics may also enhance the immune response, reduce serum cholesterol, prevent colonic cancer, prevent dental caries, prevent ulcers due to *Helicobacter pylori*, maintain urogenital health, and ameliorate hepatic encephalopathy. Further studies are required to establish their role in these conditions.

Introduction

Increasing evidence indicates that the consumption of foods containing micro-organisms, ie probiotics, confers health benefits. Clinical trials have evaluated their use in the prevention and treatment of gastro-intestinal (GI) diseases caused by pathogenic micro-organisms or by disturbances in the normal micro-flora.[1] Probiotics are defined as "live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host".[2] The ideal probiotic is one that remains viable in the intestine, adhering to the intestinal epithelium to confer a significant health benefit.[3]

In the early 20th century, Metchnikoff[4] suggested that beneficial bacteria could be administered in order to replace harmful microbes with useful ones. The term probiotic, meaning 'for life', was first coined in the 1960s by Lilly and Stillwell.[5] In recent years, there has been a rise in both research and interest in the probiotic food concept.[6]

Gut bacteria: the health-promoting flora

It has been estimated that 100 different species (with a total bacterial population between 10^{10} and 10^{12}) are present in the human intestinal tract. The composition of the GI tract flora varies between individuals and also within the same individual during life. The GI tract contains both 'friendly' and pathogenic bacteria that exist in a complex symbiosis. Various factors such as diet, climate, ageing, medication (particularly antibiotic consumption), illness, stress, and lifestyle can upset this balance leading to diarrhoea, 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. Probiotics are possibly the most natural and safe means of maintaining this balance.[7] The organisms used in probiotics are shown in the Box.[8]

Key words
Bifidobacterium; *Clostridium difficile*; Diarrhea; Lactobacillus; Probiotics

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Box. Micro-organisms used in probiotics[8]

Bacteria

Bacilli

1. *Lactobacillus: acidophilus*, *sporogenes*, *plantarum*, *rhamnosus*, *delbrueckii*, *reuteri*, *fermentum*, *lactus*, *cellobiosus*, *brevis*
2. *Bifidobacterium: bifidum*, *infantis, longum*, *thermophilum*, *animalia*
3. *Propionibacterium*

Cocci

1. *Streptococcus: lactis*, *cremoris*, *salivarius*, *intermedius*
2. *Leuconostoc*
3. *Pediococcus*
4. *Enterococcus*
5. *Enterococcus faecium*

Yeast and moulds

Saccharomyces cerevisiae, *Aspergillus niger*, *Aspergillus oryzae*, *Candida pintolopesii*, *Saccharomyces boulardii*[8]

益生菌治療腹瀉的近期研究進展

近年來，探討益生菌和含益生菌食物的研究越來越多，而其對健康的影響也越受關注。乳酸桿菌屬和比菲德氏菌是常見益生菌類，而乳酪和發酵乳「克菲爾」是含有益生菌的常用食物。益生菌一般用作治療包括因細菌、病毒和原生動物感染；輻射和抗生素療法等引致的腹瀉。各類研究皆發現益生菌也許能提高免疫反應、減少血清膽固醇、並預防大腸癌、蛀牙和幽門螺旋菌造成的潰瘍，也有助尿殖健康和改善肝性腦病。有需要進一步研究益生菌對上述疾病的治療角色。

Of the above, the most important genera are: *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Bifidobacterium*.[9] The most commonly used probiotics are lactobacilli and bifidobacterium[10]; yoghurt and kefir are foods containing probiotics which are often used to manage diarrhoea.[9]

The mechanisms by which probiotics exert their effect on the gut have attracted much interest. In order to combat GI infection, a probiotic must be non-pathogenic and must act against pathogens by different mechanisms from antibiotics—for example, by competition. They should have a fairly rapid onset of action and survive the challenges of gastric acid, bile, or concurrent antibiotics. If they modify immune processes, they may be able to destroy the invading organism. *Saccharomyces boulardii,* lactobacilli, enterococci and bifidobacteria have these properties.

A few live organisms have been used in many trials.[11] A meta-analysis suggests that probiotics can be used to prevent antibiotic-associated diarrhoea. *Saccharomyces boulardii* and lactobacilli have the potential to be used in this situation. *Saccharomyces boulardii* is a non-pathogenic yeast, with an incubation temperature of 37°C, that rapidly colonises the bowel, does not alter the normal gut flora, and is cleared from the colon after treatment is discontinued.[11] Of four yeast trials, two studies demonstrated strong benefits[12-14] but one did not.[15] Differences in the dose and duration of treatment with *S boulardii* and variations in the period of follow-up may explain this disparity. Interestingly, *S boulardii* can also destroy the receptor site for *Clostridium difficile* toxin A and B by producing a protease.[16] This may explain how *S boulardii* was able to reduce the frequency of toxin B positivity.

Lactobacillus is a beneficial bacterium because it aids the host with both the digestion and absorption of nutrients. Lactobacilli are Gram-positive, non–spore-forming, microaerophilic rods, which produce abundant lactate that lowers the pH of the intestine when sugar is converted to lactic acid, limiting the growth of certain enteropathogens (eg *Salmonella* spp).[1] *Lactobacillus* spp appear to be able to rapidly colonise the intestinal epithelia of vertebrates, depriving pathogens of attachment sites. Lactobacilli have also been shown to have immunoregulatory roles by increasing macrophage activity and enhancing the production of immunoglobulins (eg immunoglobulin A). Recent research into the molecular status of faecal samples in different animal species suggests that *Lactobacillus* populations may be unique to each individual. In some patients, the *Lactobacillus* population is very simple, containing only one to two strains, whereas others have more complex populations, with as many as 11 different strains, none of them predominant.[17] Over 70 different species of *Lactobacillus* have been identified to date; of these, only 34 have been identified at species level.[18] *Lactobacillus acidophilus, L salivarius, L plantarum, L fermentum*, and *L brevis* are the most frequently used species of lactobacilli.[19]

Enterococcus spp are Gram-positive, facultative anaerobic cocci of the Streptococcaceae family. They are spherical to ovoid in shape and occur in pairs or short chains. Enterococci are catalase-negative, non–spore-forming, and usually non-motile. These bacteria are commonly found in the intestinal microflora of both humans and animals. *Enterococcus faecium* SF68 is a specific probiotic strain that has been used in the management of diarrhoeal illnesses.

Bifidobacterium spp are Gram-positive, anaerobic rods that form a 'Y' or bifid form. These bacteria can produce acetic acid and lactic acid that lowers the intestinal pH and competitively inhibits the colonisation of enteropathogens. *Bifidobacterium* also counteracts the disturbances identified in the intestinal microflora after antibiotic therapy and produces certain B vitamins. Each human host has a unique bifidobacteria pattern.[18] The species most commonly used as probiotics are *Bifidobacterium animalia, B longum, B bifidum*, and *B infantis*.[19]

Propionibacterium freudenreichii are non-motile, non-sporulating Gram-positive short rods. They assume involution forms in acidic media or under aerobic conditions.[20]

Leuconostoc species are epiphytic bacteria that are widespread in the natural environment. *Leuconostoc mesenteroides* is a facultative anaerobe requiring complex growth factors and amino acids.[21,22] Most strains appear as cocci in liquid cultures, occurring singly or in pairs or short chains, however their morphology can vary with growth conditions; cells grown in glucose or on solid media may have an elongated or rod-shaped morphology. The cells are Gram-positive, non-motile, and asporogenous.[23]

Probiotics and management of diarrhoea

Aetiology of diarrhoea

There are several causes of diarrhoea, including

infections, radiation, antibiotic therapy, and tube feeding.[24] Standard diagnostic laboratories can identify the pathogens in approximately one third of cases of acute diarrhoea in older children and adults. Bacterial agents are responsible for approximately 10% of diarrhoeal illness in industrialised countries.[25] Viruses (especially rotaviruses) are more important in infants and cause community food- and water-borne gastroenteritis (noroviruses) in all age-groups. In most settings protozoa are uncommon causes of acute diarrhoea. In developing countries, bacterial enteropathogens, particularly enterotoxigenic *Escherichia coli*, cause just under half of the cases of endemic paediatric diarrhoea and approximately half of all cases of diarrhoea among international travellers to those areas.[26,27] In the last 3 years, newer putative agents have been identified in cases of acute diarrhoea.

Infective diarrhoea

Probiotics have been best researched as a form of management for acute infantile diarrhoea. Rotavirus is the leading cause of this condition worldwide. The primary treatment for this is rapid oral rehydration.[28] At least three systematic reviews have shown that use of probiotics achieves overall reductions in the duration of diarrhoea ranging from 17 to 30 hours.[29-31] Many mechanisms of action explaining how lactobacilli reduce the duration of rotavirus-induced diarrhoea have been proposed. These include competitive blockage of receptor sites when lactobacilli bind to receptors; signal(s) from lactobacilli regulating secretory and motility defences designed to remove perceived noxious substances; enhancement of the immune response; and production by lactobacilli of substances that inactivate the viral particles. A meta-analysis by Huang et al[32] found that when a probiotic was used at the onset of diarrhoea, the duration of acute infection was decreased by 1 day. It is not clear whether the routine use of probiotics in acute diarrhoeal illnesses is justified, as most acute diarrhoeal illnesses are self-limited. There is not much information indicating whether probiotics reduce the serious complications of diarrhoeal illnesses. Furthermore, the data do not provide a clear understanding of the type, dose, or duration of probiotic treatment required for achieving clinical benefit. Limited data suggest that the minimal effective dose in children is 10 billion colony-forming units given within the first 48 hours. Oberhelman et al[33] evaluated the use of Lactobacillus GG (LGG) as a prophylactic agent against diarrhoea in children. The regular administration of a daily dose of LGG, 6 days a week for 15 months, achieved a low rate of diarrhoea, but only in non–breast-fed infants. The use of probiotics as prophylaxis against diarrhoea is an interesting concept that remains unproven.

The data on the use of probiotics to manage adults with infective diarrhoea are limited and mixed. Allen et al[29] reviewed 23 papers on the treatment of infectious diarrhoea with probiotics and concluded that they appear to be a useful adjunct to rehydration therapy when managing both adults and children.

Antibiotic-associated diarrhoea

A variety of probiotics have been evaluated for their efficacy as a means of treating and preventing antibiotic-associated diarrhoea. At least two systematic reviews suggest that probiotics (including various bacterial species and the yeast *S boulardii*) effectively reduce the incidence of diarrhoea in patients who are taking antibiotics. A careful meta-analysis by D'Souza et al[34] found that probiotics were more effective than placebo. As probiotics have not been shown to cause harm, this use is important and cost-effective. But use of probiotics for all cases of antibiotic-related diarrhoea cannot be justified. It is also not clear which probiotics should be used and in what doses.[28]

Diarrhoea caused by *C difficile* is a common problem associated with antibiotic use. This organism is found in small numbers in the healthy intestine but disruption of the indigenous microflora by antibiotic treatment leads to an increase in their number and toxin production, leading to diarrhoea. Treatment with metronidazole or vancomycin is usually effective, but recurrences are common. Probiotics have been proven useful for both preventing and treating antibiotic-associated diarrhoea. A daily dose of LGG has been shown to be an effective means of controlling diarrhoea.[35] A study of the efficacy of a lactobacillus preparation for preventing ampicillin-associated diarrhoea in adult patients found the incidence of diarrhoea in the group taking a placebo was significantly greater.[36]

Clostridium difficile causes nearly 25% of cases of antibiotic-associated diarrhoea, making it the most commonly identified and treatable pathogen found in this condition. *Clostridium perfringens, Staphylococcus aureus, Klebsiella oxytoca, Candida* spp and *Salmonella* spp are implicated less frequently. Most mild cases of antibiotic-associated diarrhoea are due to non-infectious causes such as a decrease in the breakdown of primary bile acids and reduction in the metabolism of carbohydrates; an allergic response to, or toxic effects of, the antibiotic on the intestinal mucosa; and a pharmacological effect on gut motility.[37]

Clostridium difficile–associated diarrhoea

The antibiotics that cause *C difficile*–associated diarrhoea (CDAD) most often are clindamycin, cephalosporins, ampicillin, and amoxicillin. Probiotics

have been used to manage people with recurrent diarrhoea.[37] *Clostridium difficile* has been associated with symptomatic diarrhoea since being identified as the pathogen responsible for pseudomembranous colitis. Patients have been treated successfully with faecal enemas, providing evidence that establishing a normal bacterial flora can suppress the growth of *C difficile*.[38] We can conclude that probiotic therapy suppresses *C difficile* infection in a similar way. Biller et al[39] 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.[40]

Total flora replacement or faecal bacteriotherapy 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. Widespread application of such a strategy is unlikely due to lack of acceptability and the possibility of transmission of communicable disease. The mechanism by which faecal bacteriotherapy works, apart from 'reconstitution of a normal flora', cannot be measured as the stool contains hundreds of bacterial species. It is an adjunctive therapy in sporadic clinical use. Administration of donor stool via a nasogastric tube in 18 cases of recurrent CDAD, leading to benefit in 15, has been reported.[41]

Radiation-induced diarrhoea

Radiation to the pelvis often induces diarrhoea. Two studies have reported beneficial results with use of probiotics for radiation-induced diarrhoea.[42,43] All improved the patients' status and the authors felt that probiotics were warranted and successful as a means of decreasing radiation-induced diarrhoea. These findings needed to be studied more thoroughly.

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 travellers' diarrhoea 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 diarrhoea.

Other conditions with diarrhoea

Three trials have reported that treatment with *S boulardii* leads to a decrease in the duration of diarrhoea induced by tube feeding. Two non-blinded trials proposed that lactobacilli might have some efficacy against small intestinal bacterial overgrowth, but *S boulardii* was found ineffective in a randomised controlled trial. Elmer et al[44] reported that high doses of *S boulardii* may be effective in some subjects with human immunodeficiency virus–related chronic diarrhoea; however, further evaluation is necessary. Though there have been reports that lactobacilli are beneficial for controlling infectious diarrhoea, their use in immunosuppressed or critically ill patients is not advised, as these people are at increased risk of developing infections due to lactobacilli.[18]

In order to predict both the positive effects and the side-effects of probiotics, better knowledge of their survival within the GI tract, translocation and colonisation properties, and the fate of their active components is needed. Lactic acid bacilli display a wide range of natural antibiotic susceptibilities and resistances. Antibiotic resistance is not transmissible (with the exception of enterococci), but represents an intrinsic characteristic of the organism. Nonetheless, it is always advisable to consider safety implications. Strains resistant to clinically important antibiotics should not be used as probiotics. Checking the ability of a proposed probiotic strain to act as a donor of antibiotic resistance genes may therefore be a prudent precaution.[45]

Inflammatory bowel disease

Probiotics stabilise the immunological barrier in the gut mucosa by reducing the generation of proinflammatory cytokines. Probiotic species have shown promise in the treatment of ulcerative colitis in small studies. *Escherichia coli, Bifidobacteria*, and *L acidophilus* have been tried in the treatment of ulcerative colitis. Use of probiotics to treat Crohn's disease has yielded mixed results.[28]

Irritable bowel syndrome

Patients with irritable bowel syndrome have constipation or diarrhoea along with abdominal pain. Five randomised studies using probiotics to manage irritable bowel syndrome have been carried out, three of which reported a definite decrease in symptoms.[28]

Prebiotics

Prebiotics are defined as food ingredients that promote the growth or activity of a limited number of bacterial species for the benefit of the host. Simply put, prebiotics are food for the bacterial species considered beneficial for the host's health and well-being. The bacterial species attracting most interest are those in the *Lactobacillus* and *Bifidobacterium* genera. These broad bacterial groups do not contain all the documented probiotic strains but the defined probiotic bacteria are not necessarily excluded from those microbes with the ability to use prebiotic fibre as a substrate. More precisely, the term prebiotic refers to substances that have undergone extensive in-vitro and in-vivo testing across a number of subject species, including human beings, to confirm prebiotic fermentation characteristics. Furthermore, the fermentation characteristics making a substance prebiotic include nutraceutical effects that extend beyond those of regular nutrition. Most such effects are associated with optimal colonic function and metabolism, such as an increase in the expression or change in the composition of short-chain fatty acids, increased faecal weight, a mild reduction in luminal colon pH, a decrease in nitrogenous end products and reductive enzymes, an increased expression of the binding proteins or active carriers associated with mineral absorption, and immune system modulation. These changes in the colonic microbiota, their products, and their effects on host biochemistry and histology form the rationale for associating health benefits with the use of prebiotic ingredients. The human diet may contain a variety of foods providing fermentable fibre. Localised fermentation is crucial to the trophic needs of colonocytes, which derive the majority of their nourishment from this source. Many substances and foods have been declared prebiotic, based on fermentability alone. Various whole foods are also being considered 'prebiotic' because they contain a mixture of fibres, some of which are fermentable. This information is imprecise and confusing. Because foods are complex items with broad metabolic and nutritional effects, they should not be categorised as narrowly as the prebiotic definition demands. Furthermore, all macronutrients and their partially digested remnants arriving in the colon are available as microbial substrates, yielding a variety of fermentation products of a highly mixed nature, which may be either potentially beneficial or detrimental to health. Therefore, the word prebiotics must always refer to specific, defined substances that exhibit a particular, scientifically observed effect. Fibre, particularly fermentable fibre, is crucial for good health, but prebiotics are specific ingredients targeting specific bacteria, their fermentation end products, and potential host benefits.[46]

Conclusion

Probiotics are fast emerging as an alternative to conventional antimicrobial therapy, as they are relatively cheap and are less likely to induce resistance due to their multifaceted mechanisms of action. Besides their role in the management of diarrhoea, there is evidence that probiotics also enhance the immune response, reduce serum cholesterol, prevent colonic cancer and dental caries, reduce *Helicobacter pylori* infection, decrease urinary tract infections and hepatic encephalopathy apart from their proven efficacy in diarrhoea. Further studies are needed to establish their place in the management of these conditions.

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