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Probiotics, antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in humans

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Probiotics are living organisms which, when ingested, have a beneficial therapeutic effect. Examples are bacteria, especially *Lactobacillus rhamnosus* GG, and the yeast *Saccharomyces boulardii*. Controlled trials indicate a benefit of both of these in the prevention of antibiotic-associated diarrhoea. Other less effective probiotics are Lactinex, *Enterococcus faecium* and bifidobacteria. In the difficult clinical problem of recurrent *Clostridium difficile* disease, *S. boulardii* as an adjunct to antibiotics has shown benefit in controlled trials. There is, however, less convincing evidence for the efficacy of *Lactobacillus* GG in this disease. Additional controlled trials and safety studies are needed before there can be a widespread endorsement of probiotics for these two conditions.

Key words: probiotics; antibiotic-associated diarrhoea; recurrent *Clostridium difficile*; *Lactobacillus rhamnosus* GG; *Saccharomyces boulardii*; *Enterococcus faecium*; Lactinex.

The normal gut flora possesses a quality called colonization resistance, which prevents the overgrowth of pathogens; some of these antibacterial effects may be caused by volatile fatty acids and a decrease in pH of the luminal contents. The most common disruption of the flora is that caused by antibiotics that can cause diarrhoea (antibiotic-associated diarrhoea, or AAD), and an appealing area for the use of probiotics is in the prevention of AAD. The concept of probiotics dates back to 1908, when Eli Metchnikoff, a Nobel Prize winner, suggested, as a theory to explain why some Russian peasants lived so long, that 'ingested lactobacilli can displace toxin producing bacteria, promoting health, prolonging life'.[1] The term 'probiotic' was first used by Fuller, revised in the veterinary literature as describing a 'live microbial feed supplement which beneficially affects the host animal by improving its microbial balance'.[2] A more recent definition is 'a mono- or mixed culture of live microorganisms which, applied to animal or man, affects beneficially the host by improving the properties of the indigenous microflora'.[3]

The characteristics of an effective probiotic have been defined by Saavedra[4] as resistance to digestion by enteric or pancreatic enzymes, gastric acid and bile and an

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ability to prevent the adherence, establishment and/or replication of pathogens in the gastrointestinal tract.

ANTIBIOTIC-ASSOCIATED DIARRHOEA

AAD varies in incidence but can occur in up to 39% of hospitalized patients receiving antibiotics.[5] Broad-spectrum antibiotics (ampicillin and amoxicillin, cephalosporins and clindamycin) are most commonly implicated, probably because of a more profound alteration in the normal colonic flora.[6] Although the pathophysiology underlying this is not fully understood, it is postulated that the altered faecal flora results in changes in colonic carbohydrate digestion, decreased short-chain fatty acid absorption and an osmotic diarrhoea. In some, the pathogen *Clostridum difficile* grows in large numbers and produces toxins that cause colonic damage. When osmotic tube feeds are given in conjunction with antibiotics, diarrhoea may be even more likely. The consequences of AAD include a longer hospital stay (8 days on average), a higher cost of care ($2000–4000 per stay), a fivefold increase in the incidence of other nosocomial infections and a threefold increase in mortality (ranging from 0.7% to 38%).[5]

Probiotics in the prevention of antibiotic-associated diarrhoea

Both bacteria and yeast have been studied in the prevention of AAD (Table 1); these include the bacteria *Lactobacillus* spp. (*Lactobacillus rhamnosus* GG and a commercial preparation of lactobacilli called Lactinex®), an *Enterococcus* and the non-pathogenic yeast *Saccharomyces boulardii*. A recent meta-analysis of probiotics in the prevention of AAD evaluated nine controlled trials whose results indicated that both lactobacilli and *S. boulardii* have the potential to prevent AAD.[7] One recent study supporting these findings is a controlled trial of three different probiotic regimens to prevent diarrhoea associated with anti-*Helicobacter pylori* therapy; this showed a rate of diarrhoea of 5% with all three probiotic regimens (*Lactobacillus* GG, *S. boulardii*-and a mixture of *Lactobacillus acidophilus* and *Bifiidobacterium lactis*), compared with 30% with placebo ($P <$).[8]

Table 1. Evidence for the action of probiotics in antibiotic-associated diarrhea.

Probiotic	Efficacy	Quality of evidence
Bacteria		
Lactobacillus GG	Very good	Good (several controlled trials); one negative
Latinex	Fair	Moderate
Enterococcus SF68	Fair	Poor
Bifidobacteria	Fair	Small trials only
Yeast		
Saccharomyces boulardii	Very good	Several controlled trials; one negative

Specific probiotics

Lactobacilli

Lactobacillus rhamnosus strain GG was isolated by Gorbach and colleagues in 1987.[9] It tolerates both bile and gastric acid and survives passage through the gastrointestinal tract, persisting for days.[10] It has been shown to adhere to small intestinal cells and elaborates an antimicrobial substance. In the US, it is marketed as Culturelle, a dietary supplement, and it is available in fermented milk form in Finland and some European countries. There have been several trials of the efficacy of *Lactobacillus* GG in decreasing or preventing AAD, with fewer days of diarrhoea and fewer diarrhoeal stools per day. In adults given erythromycin, there was less diarrhoea in those taking *Lactobacillus* GG yoghurt than in to controls.[11] In two studies, children given a range of oral antibiotics for respiratory infection suffered less AAD.[12,13] A controlled trial of *Lactobacillus* GG to prevent antibiotic-associated symptoms in 120 asymptomatic patients receiving antibiotics against *H. pylori* infestation showed a significant reduction in diarrhoea as well as in the symptoms of bloating and taste disturbance.[14] However, a recent study of 267 patients on antibiotics randomized to *Lactobacillus* GG or placebo failed to show any decrease in the incidence of diarrhoea-29% of both groups developed the symptom.[15]

The commercial preparation Lactinex, is a mixture of *L. acidophilus* and *L. bulgaricus*, has also been studied. It has not, however, been shown to have convincing efficacy in the prevention of AAD. In a study of hospitalized adults taking amoxicillin, the occurrence of AAD was 8% with Lactinex, compared with 21% with placebo, which was not a significant difference.[16] In a separate study of children given amoxicillin, there was no benefit with Lactinex, although the number involved was small.[17] One author suggested that the variability in batches of probiotic would explain its only modest efficacy as neomycin-treated patients demonstrated less diarrhoea when treated with one batch but not the other.[18] This illustrates one difficulty of working with living organisms, that of quality control. Other problems are differences between strains, and between patients and antibiotics studied.

Enterococcus faecium

Enterococcus faecium SF68 is marketed as Bioflorin. The organism is found in healthy adults, produces lactic acid, survives in a low-pH environment, resists antibiotics and inhibits pathogens. Despite these appealing features, it has shown only modest efficacy in the prevention of AAD in two controlled clinical trials. The rate of AAD was 9% with SF68 compared with a placebo rate of 27% when studied with various antibiotics[19], and 3% compared with 18% for placebo when used with drugs to treat pulmonary tuberculosis.[20]

Bifidobacteria

Bifidobacteria are bacterial that are found in the newborn intestinal tract after birth, amounts being seen in breast-fed infants. They are thought to be beneficial. Two studies of their use as a probiotic to prevent clindamycin diarrhoea and erythromycin diarrhoea have shown same benefit.[21,22] In the clindamycin study, 10 volunteers taking a fermented milk with *B. longum* and *L. acidophilus* had less gastrointestinal discomfort.[21] *Bifidobacterium longum* was studied in 10 healthy volunteers receiving erythromycin, with a significant reduction in stool weight and frequency.[22] Additional trials of bifidobacteria are, however, needed.

Yeast

The non-pathogenic yeast *S. boulardii* was discovered in south-east Asia in the 1920s. It has an optimal growth temperature of 37 °C and survives passage through the gastrointestinal tract. Three controlled trials have demonstrated its efficacy in the prevention of AAD.

A study of 388 French outpatients given 200 mg *S. boulardii* daily showed an AAD rate of 4% (nine of 99) compared with 17% (33 of 99) in those receiving placebo ($P < 0.01$).[23] A U.S. study of 180 hospitalized patients receiving various antibiotics showed a diarrhoea rate of 9% (11 of 116) with *S. boulardii* (250 mg twice daily) compared with 22% (14 of 64) with placebo.[24] Another U.S. study of 193 patients on β-lactam antibiotics reported AAD rates of 7% (seven of 97) with *S. boulardii* (500 mg twice daily) and 15% (14 of 96) with placebo.[25] A placebo-controlled trial of *S. boulardii* in critically ill, tube-fed patients in intensive care units showed a significant reduction in the incidence of diarrhoea, antibiotics being a significant risk factor.[26] A British study of 69 elderly inpatients receiving antibiotics failed, however, to demonstrate any efficacy of *S. boulardii* (113 g twice daily) in the prevention of AAD.[27]

In summary, the action of probiotics in preventing of AAD varies from very good (*S. boulardii*, *Lactobacillus* GG, *B. longum* and *B. longum* with *L. acidophilus*) to moderate (Lactinex, Enterococcus SF68).

Practice points

- diarrhoea is a frequent side-effect of antibiotics, occurring in up to 29% of hospitalized patients although fewer outpatients
- some probiotics given with antibiotics can significantly reduce the incidence of AAD in controlled trials: *Lactobacillus* GG and *S. boulardii* are best supported by controlled trials and by a meta-analysis of probiotic use in the prevention of AAD
- before probiotics can be adopted for widespread use, high-risk populations should be identified and cost–benefit analyses and adequate safety studies performed

RECURRENT *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DISEASE

Clostridium difficile is a Gram-positive anaerobic bacteria that produces two toxins, an enterotoxin A and a cytotoxin B, which causes colitis, usually as a result of antibiotic therapy. Treatment with metronidazole or vancomycin is effective, but the disease recurs after therapy in a small number of individuals. In this situation, multiple recurrences are common and can be difficult to treat.

The pathophysiology is not known but probably results from the inability of the normal flora to repopulate and suppress *C. difficile* overgrowth. In studies, both the reacquisition of and reinfection with *C. difficile* have been documented. One suggestion is that spores are a source of recurrent *C. difficile* infection, but data to prove this are lacking. One study in fact showed no correlation between the number of spores in the stools after therapy and the risk of subsequent recurrent *C. difficile* disease.[28] Risk factors for recurrent *C. difficile* disease include intercurrent or continued antibiotics, renal disease, elderly age and female sex.[29,30] Retreatment with antibiotics is usually necessary, pulsed and/or tapered doses improving efficacy.[28] Because an alteration in

Table 2. Probiotic therapy in the treatment of recurrent *Clostridium difficile*-associated disease.

Probiotic	Efficacy	Strength of evidence
Lactobacillus GG	Fair	Moderate; small trials, none controlled
Saccharomyces boulardii	Good	Very good; several controlled trials
Saccharomyces cerevisiae	None	Anecdotal
Faecal enema and rectal therapy	Mild	Anecdotal

the faecal flora is a factor in pathophysiology, the use of probiotics is appealing. Probiotics tested to prevent recurrent *C. difficile*-associated diarrhea (CDAD) include bacteria and yeast (Table 2).

Probiotics in the prevention of *Clostridium difficile*-associated diarrhoea

Bacteria

Early uncontrolled trials of *Lactobacillus* GG suggested its possible efficacy in recurrent CDAD. In three different studies, 8 of 11 adults[31], 2 of 4 children[32] and 5 of 9 adults[33] were cured. A preliminary report of a controlled trial suggested that *Lactobacillus* GG[34] was effective, but unfortunately no final results have been published.

In an effort to repopulate the normal colonic flora, investigators have used several approaches. The most appealing is a rectal instillate of micro-organisms, approximately 10 species of aerobes and anaerobes, showing the important role of *Bacteroides* spp.[35] Less aesthetically appealing are studies of stool transfusion.[36,37] A recent report of donated stool administered at colonoscopy showed efficacy in two women.[38] All published studies document only a small number of participants, and no controlled trials have been performed.

Yeast

Saccharomyces boulardii

Early animal work in the hamster model of recurrent *C. difficile* infection showed an efficacy of *S. boulardii*.[39] An early uncontrolled unblinded or open trial of *S. boulardii* (500 mg twice daily) showed efficacy in recurrent CDAD in adults (11 of 13, 85%).[40] An open trial of *S. boulardii* (640 mg three times daily) against recurrent *C. difficile* in seven patients with renal failure showed an improvement in five patients.[41] Two controlled trials in adults showed efficacy compared with placebo. In one study, recurrent CDAD occurred in 9 of 26, (34%) receiving *S. boulardii* (500 mg twice daily) compared with 22 of 34, (64%) of those taking a placebo ($P < 0.05$).[42] A later trial of 168 patients showed a similar efficacy in a subset of 3 of 18 (17%) being treated with *S. boulardii* (500 mg twice daily) compared with 7 of 14 (50%) receiving placebo, ($P < 0.05$), but this was significant only in patients treated with high-dose (2 g per/day) vancomycin and not with lower doses of vancomycin or metronidazole.[43]

Saccharomyces cerevisiae

Saccharomyces cerevisiae is the active ingredient in commercial Baker's yeast. One case report of a woman with recurrent CDAD described the effectiveness of this

preparation after several recurrences of disease[44], but there have been no controlled trials involving this agent. The yeast appears to be different from *S. boulardii*[45]; *S. boulardii* has recently been reclassified into the species *S. cerevisiae*[46], but the taxonomic assignment remains controversial.[47]

Practice points

- *Clostridium difficile* disease responds well to treatment with metronidazole or vancomycin but recurs after treatment in a small number of cases, with repeated recurrences that are difficult to treat
- probiotics are appealing for the treatment of recurrent CDAD as normalization of the faecal flora is important to prevent a continued overgrowth of *C. difficile*
- controlled trials of *S. boulardii* show benefit in the treatment of recurrent CDAD. Evidence for efficacy with *Lactobacillus* GG is, however, less convincing
- no single probiotic is uniformly effective; additional agents with proven efficacy and good safety profiles are needed

MECHANISM OF ACTION OF PROBIOTICS

The mechanisms of action of probiotics are not clearly understood. Possible mechanisms include a stimulation of immunity, competition for nutrients, an inhibition of epithelial and mucosal adherence of pathogens and the production of antimicrobial substances.[48] Additional mechanisms may include receptor competition, effects on mucin secretion and the immunomodulation of gut-associated lymphoid tissue.[49]

Lactobacillus GG has been shown to adhere to mucosal cells; it can compete for nutrients and produces a substance that inhibits bacteria[50] and changes in faecal β-glucuronidase, which may be a marker of intestinal bacterial metabolism.[10] Others have shown that it augments the local immune response, especially the IgA response, to rotavirus.[51] In a rat model of cow milk challenge, *Lactobacillus* GG reversed the increased intestinal permeability that had been induced.[52] Competition for receptor sites between *Lactobacillus* GG and pathogens has been studied in Caco-2 cells and suggests steric hindrance.[53] There is a report of a prevention of cytokine-induced apoptosis in intestinal epithelial cell models, suggesting a possible increased survival of intestinal cells.[54] *Lactobacillus* GG has also been known to induce the production of nitric oxide[55], which could affect mechanisms of diarrhoea.

Saccharomyces boulardii produces a protease that inactivates the toxin A receptor in animals, produces increased levels of secretory IgA and IgA antitoxin A, and competes for attachment sites in rabbit ileum.[56,57] The micro-organism also blocks cholera-induced secretion in rat jejunum.[58] Stimulation of the IgA immune response to *C. difficile* toxin A has been documented in mice[59]; *S. boulardii* has also been shown to block *C. difficile* adherence to cells in vitro[60], possibly as a steric hindrance effect.

AREAS FOR FURTHER STUDY

This is a fertile area for further study, especially in terms of the identification of newer agents, studies of efficacy, mechanical studies, pharmacokinetic studies and further

evaluation of the interplay with normal flora.[61,62] It will be important to identify high-risk populations with AAD who would benefit the most from probiotic therapy as an adjunct to antibiotics.

SUMMARY

Probiotics are appealing because, as living organisms, they have multiple mechanisms for survival that may benefit the host. Controlled trials and meta-analyses indicate an efficacy of *Lactobacilli* GG and *S. boulardii* in the prevention of AAD. Controlled trials demonstrate the effectiveness of *S. boulardii* as an adjunct in the treatment of recurrent CDAD. No probiotics have uniform efficacy, but overall, their safety profile is good. Alternatives to antibiotics are appealing, but adequate clinical trials are crucial before widespread use is recommended.

Research agenda

- probiotics need to be studied in well-controlled trials of efficacy, with adequate safety studies
- populations at high risk of AAD should be identified before the widespread use of probiotics is undertaken
- the pathophysiology underlying and risk factors for recurrent CDAD need to be identified
- the use of probiotics in recurrent CDAD needs to be better defined, as must their role as an adjunct to antibiotics

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