

Probiotics in the prevention and treatment of antibiotic associated diarrhoea

PREBIOTICS /
PROBIOTICS

ANTIBIOTIC ASSOCIATED DIARRHOEA (AAD)

The microbiota in our intestines is a fragile ecosystem. In a 'healthy' person the approximately 10^{14} bacteria in the intestines live in balance. However, this balance can easily be disturbed by stress factors, like the use of antibiotics.

Antibiotics are indispensable for the treatment of several diseases. Since antibiotics can destabilise the natural intestinal microbiota, the use of antibiotics can result in unwanted side effects such as antibiotic associated diarrhoea (AAD).

Antibiotic associated diarrhoea is defined as "diarrhoea developing a few hours after onset of antibiotic therapy to 6-8 weeks after antibiotic discontinuation" (1). The incidence of antibiotic associated diarrhoea differs from 5-39% (2) depending on the type of antibiotic used (broad spectrum antibiotics are most commonly implicated), the definition used for diarrhoea and on host factors.

The consequences of AAD in care-taking facilities include a longer stay, a higher cost of care, an increase in the incidence of other nosocomial infections and an increase in morbidity and mortality (2). To complicate the situation, it should be noted that resistance against antibiotics is an increasing risk factor for which no good pharmaceutical alternative is available.

Causes of AAD

Although the pathophysiology underlying the mechanism of AAD is not fully understood, the cause of AAD may be due to direct toxic effects of antibiotics on the intestine, pharmacological effect on the intestinal motility and direct effects on immune-cell function. However, most cases of AAD are thought to be due to disturbances of the intestinal microbiota which is associated with loss of

colonisation resistance, altered metabolic function and overgrowth of pathogenic bacteria (1).

Pathogenic micro-organisms associated with AAD are: *Clostridium perfringens*, *Klebsiella oxytoca*, *Staphylococcus aureus*, *Candida* spp., and *Salmonella* spp. (1). Moreover, 12-25% of AAD is caused by overgrowth of *Clostridium difficile* (3,4) which can cause serious complications like pseudomembranous colitis.

Treatment of AAD

Managing antibiotic associated diarrhoea depends on the clinical presentation and the inciting agent used (5,6). In mild to moderate diarrhoea conventional methods that are applied include rehydration, discontinuation of the inciting agent or replacement by an antibiotic with a lower risk of inducing diarrhoea. When severe or persistent diarrhoea occurs, the challenge is to identify the cause of this problem. Diarrhoea is usually caused by the loss of important metabolic functions and the loss of the colonization resistance of the normal microbiota. However in some cases the severe or persistent diarrhoea is caused by the overgrowth of a pathogen.

C. difficile is the most commonly seen pathogen and can be treated well. In 22% cases of diarrhoea related to *C. difficile*, withdrawal of the inciting agent will lead to resolution of the clinical signs in three days (7).

In other cases the treatment of *C. difficile* associated diarrhoea involves oral metronidazole or oral vancomycin for 10 days. Diarrhoea usually resolves in two or three days. However, approximately 20% of patients with *C. difficile* infection will relapse. Most of them will be treated with another course of metronidazole or vancomycin, but 5% will experience more relapses. The

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management of these chronic cases remains controversial (7).

The increase in antibiotic resistance of micro-organisms makes alternative treatments more attractive. Living micro-organisms that can mimic the normal functions of the intestinal commensal microbiota could offer a solution. These micro-organisms are called 'probiotic' bacteria and products containing these beneficial bacteria are called 'probiotics'.

PROBIOTICS

A definition of a probiotic is 'a live microbial food supplement which beneficially affects the host by improving its microbial balance' (8). A definition generally used at present was proposed by the FAO/WHO in 2002: 'live micro-organisms which, when administered in adequate amount confer a health benefit to the host' (9). The characteristics of an effective probiotic have been defined by Saavedra (10) as:

- Resistance to digestion by enteric or pancreatic enzymes, gastric acid and bile;
- Ability to prevent the adherence, establishment and/or replication of pathogens in the gastrointestinal tract.

Probiotics can restore the balance of the microbiota amongst others due to their ability to adhere to mucosal cells. This inhibits growth of pathogens by competition for receptors on the epithelial cells. Probiotics can also restore the balance by competition over nutrients. In addition probiotics strengthen the non-immunological defences of the gastrointestinal tract by (11):

- Production of antimicrobial substances;
- Stimulating mucus secretion;
- Reinforcing gut barrier function (maintaining gut integrity);
- Improving gut motility.

Finally, it is also suggested that probiotics protect against enteropathogens by immunological defences (11):

- Stimulating cytokine production;
- Enhancing the phagocytic capacity of polymorphonuclear cells and macrophages;
- Augmenting natural killer helper (NKH) cell activity;
- Enhancing specific antibody responses to pathogenic organisms.

PROBIOTICS AND ADVANTAGES IN ANTIBIOTIC ASSOCIATED DIARRHOEA

Studies performed with probiotic

bacteria have studied the beneficial effects of probiotics in preventing and treating antibiotic associated diarrhoea. D'Souza *et al.* (12) have reported a meta-analysis of nine randomised double-blind trials comparing probiotics with placebo in the prevention of antibiotic associated diarrhoea. They found a combined odds ratio of 0,37 ($p < 0,001$). Their results suggested that probiotics are useful in the prevention of diarrhoea. Cremonini *et al.* (13) also conducted a meta-analysis which included seven studies. The combined relative risk found was 0,40 ($p < 0,05$). This also suggested that there is a strong benefit of probiotic administration on antibiotic associated diarrhoea.

Recently a new meta-analysis of McFarland (14) showed that three types of probiotics show promise as effective therapies for the prevention of antibiotic associated diarrhoea (RR=0.43, 95% CI 0.31, $p < 0.001$). Probiotic mixtures are also one of these three types. Trials were included in which specific probiotics were given either to prevent or treat the diseases of interest. They had to be randomised, controlled, blinded efficacy trials in humans published in peer-reviewed journals. For the analysis 25 randomized trials could be used.

None of the probiotic formulations included in the meta-analysis however were specifically developed for antibiotic associated diarrhoea and so the question remains which is the most suitable probiotic formulation. One should look at the specific characteristics that probiotic bacteria need to have to be effective in the respective application. One selection criterion could for example be the inhibition of *Clostridium (in vitro)*. Besides that, different formulations have been used (monostrain and multispecies) and there are indications that a multispecies probiotic can have several advantages over a monostrain or monospecies probiotic (15).

The clinical trials with probiotics that have been performed in the past are numerous but there are a number of drawbacks that should be taken into account:

- partly paediatric patients;
- in most cases only clinical outcome was investigated, with limited bacteriology (only culture);
- difference in dose and duration between trial;
- difference in used antibiotics;
- difference in used probiotics (monostrain/multispecies).

Recently, a study with healthy volunteers was conducted at the University Hospital Maastricht with a specific multispecies probiotic formulation (Ecologic® AAD

from Winlove BioIndustries BV). The product contains 10 different probiotic strains – *Lactobacillus acidophilus* (2x), *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, *Enterococcus faecium*, *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Bifidobacterium longum* – which have been specifically selected for this application.

The selection criteria were: ability to survive the GI tract (*in vitro*), pathogen inhibition (a.o. *Clostridium*), lack to confer possible antibiotic resistance and compatibility with the other strains used in the product. In this study the effect of a specific multispecies probiotic on bacteriological, immunological and clinical parameters was studied both during as well as after antibiotic treatment. Besides diarrhoea incidence, other parameters like the alteration of the intestinal microbiota (both the composition and metabolic activity) and immunological changes were investigated.

Forty healthy volunteers were treated with 500 mg amoxicillin twice daily from day 1-7 and 5 g of the specific probiotic formulation (10^9 CFU/gr) was given twice daily from day 1-14. The first results of this study showed that bowel movements with a frequency ≥ 3 for at least 2 days and/or a consistency ≥ 5 (Bristol scale) for at least 2 days were reported less frequently in the probiotic compared to the placebo group – 48% vs. 79% ($p < 0,05$).

This showed there is a significant reduction in the diarrhoea like defecation score which can imply that the incidence of antibiotic associated diarrhoea during and after antibiotic intake due to the intake of the specific probiotic formulation can be reduced (to be published).

First results have also shown that the similarity indices of the dominant faecal microbiota were significantly higher ($p < 0,05$) after 35 days compared to day 0. This shows that there is a recovery stimulation of the intestinal microbiota with this specific formulation. Moreover, the probiotic group remained more stable after cassation of amoxicillin than within the placebo group over time. Also a significant increase in serum SIgA at day 63 in the probiotic group compared to the placebo group ($p < 0,05$). It indicates that there is a positive effect on the immune system.

This study will be followed by a clinical trial, which already started, with COPD- and asthma patients. Expectations for this study are very hopeful, especially after the good results that were found in the first trial, with the healthy volunteers.

The risk reduction in diarrhoea like

symptoms, increased stimulation of the recovery of the intestinal microbiota, and stimulation of the immune system in the probiotic group indicates that this specific probiotic formulation can theoretically offer the following advantages for the patient:

- Faster recovery from diarrhoea;
 - Less complications like *C. difficile* outbreaks;
 - Less secondary infections;
 - Lower morbidity and mortality;
 - Increased comfort/quality of live.
- Of course the advantages for care-taking facilities should be taken into account as well:
- Reduced duration of hospital stay;
 - Reduced workload on nursing staff;
 - Reduction in prescription of antibiotics;
 - Reduced antibiotic-resistance related problems;
 - Fewer ward closures for disinfection;
 - Less bed-days lost because of infection control requirements.

In today's hospital the nursing staff is overworked and it should not be underestimated how important it is to have a large reduction of AAD patients to look after.

CONCLUSIONS

Many different probiotic formulations have been tested in several studies, which were all conducted with a different setup. Moreover, some studies were not double-blind and placebo controlled.

The meta-analyses of D'Souza *et al.*, Cremonini *et al* and McFarland. (12-14) suggest that probiotics are useful in the prevention of AAD. However, in the studies included into the meta-analysis,

different probiotics were used. None of these formulations were specifically developed for this specific problem (antibiotic associated diarrhoea) and therefore there is no clear indication of which formulation acts best.

For the development of a probiotic formulation with an optimal beneficial effect, one should look at the specific characteristics that probiotic bacteria need to have to be effective in the respective application. Besides, there are indications that a multispecies probiotic can have advantage over monospecies or monostrain formulations (15).

The specific multispecies probiotic formulation that was developed and tested in the University Hospital Maastricht has been selected on different criteria and has already proved to be an effective formulation for the risk reduction of antibiotic associated diarrhoeal like symptoms. It also showed a stimulation in the recovery of the intestinal microbiota and a positive influence on the immune system. If the planned patient study shows similar results, this product will be able to offer great advantages for both patients as well as care-taking facilities.

Another study also indicates that there might be possible interesting effects of probiotics during and after antibiotic treatment on the total numbers of antibiotic-resistant strains during re-growth of the intestinal microbiota (16). In this trial, with 162 patients, the number of patients harbouring antibiotic-resistant enterococci at day 35 post therapy increased significantly ($p < 0,05$) in the placebo group. There was no change in the incidence rate of antibiotic resistance among patients in the probiotic group.

Research in this field is gaining more attention and this may hold a very interesting application of probiotics during and after antibiotic treatment for the near future.

REFERENCES

- 1) BEAUGERIE L. *Best Pract. & Res. Clin. Gastroenterol.* **2004**, *18* (2), 337-352
- 2) MCFARLAND L.V. J. *Ped. Gastroenterol. Nutr.* **1998**, *10*, 292-307
- 3) FEKETY R., *et al. Clint. Infect. Dis.* **1997**, *24* (3), 324-333
- 4) MOYENUDDIN M., *et al. Curr. Gastroenterol. Rep.* **2002**, *4* (4), 279-286
- 5) BERGOGNE-BÉRÉZIN E. *Int. Journal of Antimicrobial Agents* **2000**, *16*, 521-526
- 6) BARTLETT J.G. *The N. Engl. J. Med.* **2002**, *346* (5), 334-339
- 7) BARBUT F., MEYNARD J.L. *BMJ* **2002**, *324*, 1345-1346
- 8) FULLER R. J. *App. Bacteriol.* **1989**, *66*, 365-378
- 9) Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food; London, Ontario, Canada; April 30 and May 1 2002).
- 10) SAAVEDRA J.M. J. *Ped. Gastroenterol. Nutr.* **1995**, *21*, 125-129
- 11) GILL H. *Best Pract. Res. Clin. Gastroenterol.* **2003**, *17* (5), 755-773
- 12) D'SOUZA A.L., *et al. BMJ Clinical Research ed.* **2002**, *324* (7350), 1361
- 13) CREMONINI F. *Aliment. Pharmacol. Ther.* **2002**, *16* (8), 1461-1467
- 14) MCFARLAND L.V. *Am. J. of Gastroenterology* **2006**, *101*, 812-822
- 15) TIMMERMAN H.M. *Int. J. Food Microbiol.* **2004**, *96* (3), 219-233
- 16) S. PLUMMER. *et al. Int. J. Antimicrob. Agents* **2005**, *26*, 69-74

NutraCos news

FENCHEM ANNOUNCES EXPANSION OF LABORATORY

Fenchem's new laboratory is currently put into use, four times larger than before to meet the demand of quality assurance and research on innovative products. It provides scientific laboratories, offices, meeting rooms, library and other supporting facilities arranged in six interlinking modules.

The new laboratory not only focuses on the analysis of functional components, heavy metals, microorganism etc, but is equipped with PPSL (Pulsed Photo Stimulated Luminescence) Screening System, which is designed and developed

at Scottish Universities Research and Reactor Centre and provides an opportunity for effective detection of irradiated foods using a rapid instrumental method.

REPORT REVEALS SUCCESSFUL KID-SPECIFIC DAIRY STRATEGIES

In a new report, food nutrition and health specialist JULIAN MELLENTIN highlights the dairy industry's advancement into the latest functional food categories. Entitled *Eight Key Case Studies In Kids' Nutritional Dairy*, the paper examines leading companies' approaches to successful marketing of kids' healthy dairy products. According to the author, the kids' food market is valued at over \$ 300 billion worldwide, yet there is still an abundance of opportunities to be explored by food

manufacturers aiming healthy dairy products at kids and mothers alike. In the report, Mellentin outlines the consumer market's key health concerns and how to address them effectively.

Supported by extensive interviews, the case studies offer detailed analysis of branding, marketing and pricing strategies of kid's nutritional dairy products. Observing the evolution of the nutritional dairy market, Mellentin claims probiotics, omega-3s and calcium are the three areas in which "the most significant success stories are being made". He explains: "The dairy industry's ambition to innovate with more and more new ingredients seems to be growing increasingly bolder. No other food or beverage category has played such an important role in driving the functional food revolution in Europe, South America and Asia"