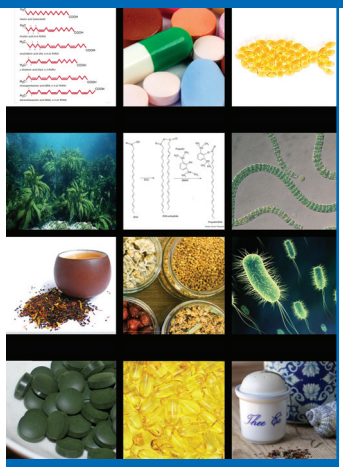

Use of a multi-species probiotic*

For the prevention of antibiotic associated diarrhea

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SUMMARY

Previous experience in our hospital and in the literature indicate an incidence of diarrhea between 5% and 50% after antibiotic treatment (depending on the antibiotic used).
In the present study, a total of 199 patients receiving antibiotic treatment after surgical intervention received additionally to their antibiotic treatment daily two sachets of the multispecies probiotic MSP. The application of MSP reduced the incidence of diarrhea to 0.5% (1 patient out of the199 probands). The probiotic administration was forgotten in two subjects, both generated AAD (with evidence of *Clostridium difficile* in one subject). In conclusion, the use of the multispecies probiotic for primary prevention of AAD was found to be successful. Prevention of AAD in this manner promises to result in a reduction of overall costs for health care.

INTRODUCTION

The human intestinal tract comprises a large and complex ecosystem with a wide variety of bacterial species that play an important role in human physiology. This intestinal microbiota is involved in colonisation resistance (i.e. preventing overgrowth of pathogens), several metabolic processes and modulates both the mucosal and systemic immune system. Each individual is thought to have a unique intestinal microbiota (i.e. 'genetic fingerprint') that is relatively stable in time in healthy persons (**1**).

Despite its stable overall composition, the adult intestinal microbiota can to be modified to a certain extent, mostly due to external factors such as antibiotic treatment, stress, change of diet, travelling, etc (**2**). These ecological disturbances may lead to a decreased colonization resistance (**3**) resulting in an increased risk for overgrowth of certain bacterial species (**4**).

Antibiotic use has been shown to disturb the intestinal microbiota as it not only affects the pathogens to which it is directed but also the indigenous microbiota, which may result in antibiotic-associated diarrhoea (AAD) during the period of administration and even up to 8 weeks following termination of treatment (**5,6**). In literature, the incidence of AAD ranges from 5-49% depending on the definition of diarrhoea, the type of antibiotic used and host factors. Clindamycin and cephalosporins are among the major causes of diarrhoea, followed by amoxicillin/clavulanic acid and other penicillins (**6**). The severity of AAD varies from cases of mild diarrhoea to severe pseudomembranous colitis which is mainly caused by *Clostridium difficile*. A moderate AAD is considered as a side effect, and is generally not treated as it usually ceases a few days after the cessation of antibiotic treatment.

It is assumed that the disturbance of the intestinal microbiota is short-term, but recently medium and long-term disturbances in (specific) bacterial populations have also been described (**7**). While AAD is partly considered as a side effect, under certain circumstances there is a risk of development of nosocomial infections, resulting in increased morbidity and mortality with prolonged hospitalization and thus an increase in health care expenditure (**6**). Furthermore, the disruption of the intestinal microbiota induced by antibiotics is also a risk factor for development of IBD (inflammatory bowel disease) (**8**) and immune deficiencies caused by re-infections.

For the past decade, probiotics have been the focus of research groups and fundamental importance has been given to the mucosal immune system (**9-12**). Research

**Omni-BIOTIC®10 AAD* (Ecologic®AAD) is a multispecies probiotic produced by *Winclove, Bio Industries*, Amsterdam, The Netherlands

has shown that probiotics restore the disrupted intestinal microbiota by influencing its composition. Particularly positive effects were obtained with antibiotics used for prevention and treatment (***Table 1***) (**13-20, 21**).

There is, however, great variation in efficacy for the different species and strains of bacteria used. A multispecies probiotic, combining a variety of probiotic properties, could therefore be more effective (**22-24**).

Table 1 *Meta analysis relative to AAD and probiotics*

Meta analysis	**Correlated studies (Type of Diarrhea)**	**Participants in the study (target group)**	**Conclusions**	**Relative Risk or Risk Ratio and confidence interval (C.I.)**
Cremonini *et al*, 2002 (**13**)	7 (AAD)	22 (all groups)	The results show that the use of probiotics is effective against antibiotic associated diarrhea (AAD). Due to the heterogeneity of the type of study and the population involved, it was not possible to come to any final conclusion.	RR 0,40 (0,27-0,57)
D'Souza *et al*, 2002 (**14**)	9 (AAD)	1212 (all groups)	The analysis shows that probiotics are effective in reducing the risk of AAD. In these cases, Lactobacillus show to have great potential. The effectiveness of probiotics in the treatment of AAD has not yet been verified.	OR 0,37 (0,26-0,53)
Huang *et al*, 2002 (**15**)	18 (Acute diarrhea, unspecified)	(Children)		OR/RR not mentioned, the use of probiotics reduced the duration of acute diarrhoea to 0.8 days ($p < 0.001$).
Van Niel *et al*, 2002 (**16**)	9 (Acute diarrhea, unspecified)	765 (Children)	OR/RR not mentioned,	the use of probiotics reduced the duration of the acute case to 0.7 days (with a reduction of the frequency of stools to 1.6 already at the second day of treatment).
Johnston *et al*, 2006 (**17**)	6 (AAD)	707 (Children)	This study shows that probiotics are effective against the risk of antibiotic associated diarrhea.	RR 0,43 (0,25-0,75)
Sazawal *et al,* 2006 (**18**)	19 (AAD) 6 (ravellers) 9 (other cases of acute diarrhea)	2050 (all groups) 1466 (all groups) 1370 (all groups)	3 types of probiotics preparations (S. boulardii, LGG and multi-species probiotics) have significantly aided the onset of antibiotic associated diarrhea.	RR 0,48 (0,35-0,65) RR 0,92 (0,80-1,06) RR 0,66 (0.47-0,92)
Szajewska *et al*, 2006 (**19**)	6 (AAD)	766 (Children)	Compared with the placebo, probiotics have been linked to a 52% relative reduction of AAD. Despite the high statistical heterogeneity, the detailed analysis has revealed significant effects of probiotic products. A structured analysis has shown the effectiveness of the multi-strain products.	RR 0,44 (0,25-0,77)
McFarland *et al*, 2006 (**20**)	25 (AAD) 6 (CDAD)	2810 (all groups) 354 (adults)	Significant therapeutic effects were shown for the "AAD Prevention" sub-group. Multistrain products gave better results.	RR 0.43 (0.31-0,58) RR 0.59 (0.41-0,85)

AAD: Antibiotic associated diarrhoea; CDAD: Clostridium difficile diarrhea; RR: Relative risk OR: Odds ratio

MATERIALS AND METHODS

Composition of the multispecies probiotic

The multispecies probiotic was developed to reduce the risk of AAD. The product consists of the following nine bacterial strains at a high dose of 109 cfu/g: *B. bifidum* W23, B. lactis W51 (formerly known as *Bifidobacterium longum* and *Bifidobacterium lactis*, due to identification these strains were indistinguishable from each other, and therefore now regarded as one strain), *E. faecium* W54, *L. acidophilus* W37 and W55, *L. paracasei* W72, *L. plantarum* W62, *L. rhamnosus* W71, *L. salivarius* W24, 5% mineral mix (potassium chloride, magnesium sulphate and manganese sulphate) and 15% of Raftilose® synergy1 (enriched with inulin and oligofructose).

This multispecies probiotic product was used in a double-blind, placebo-controlled randomized study (**25**) in which the effects on composition of the intestinal microbiota in healthy volunteers during and after amoxicillin intake were investigated. Moreover, the effect of the probiotic product on metabolic activity of the intestinal microbiota, on bowel habits and on the immune system were studied too.

Treatment protocol

In the last years, the Department of General Surgery of Landesklinikum Thermenregion Neunkirchen (Austria) observed an increase in the presence of antibiotic associated diarrhoea (AAD). The severity of diarrhoea differs from mild cases to pseudomembranous colitis induced by *Clostridium difficile*. In rare and particularly acute cases, AAD could even lead to development of a toxic mega colon. Perforations in the intestine are not rare and often require surgery. The last years there has been an increase of these serious cases. Mortality has augmented with the increase of cases of AAD and as consequence this has led to extension of medium term hospitalization with a substantial increase in health costs.

Since March 2007, all the patients in the surgery ward who were treated with antibiotics received MSP to be taken on a regular basis till June 2009; 15,000 packages were administered, corresponding to 7500 days of therapy. The data on antibiotic therapy and use of MSP were collected up to 6 months of observation (November 2008 up to May 2009) and the effects were analyzed. Diarrhoea was classified as a defecation pattern of > 3 abnormal stools per day.

During the observation period, 199 patients had been treated with antibiotics combined with MSP. Out of the 199 subjects, 104 were women and 95 were men with an average age of 68 (20-98 years old). The hospital stay was 13.9 days on average (2-56 days). Antibiotic therapy (enteral and parenteral) was implemented for on average 9.05 days (2-33 days). The average duration of administration of MSP was 10.08 days (2-33 days).

In the observation period, the following infections were treated with antibiotics: enteritis, colitis, diverticulitis, cholecystitis, peritonitis, pneumonia, pancreatitis, phlegmonous appendicitis, bronchitis, hepatic abscess colangitis, erysipelas, diabetic gangrene of the lower limbs, Helicobacter gastritis, urinary tract infections, infected ulcus cruris, abscesses, phlegmon. The antibiotics used for treatment of the different diseases (monotherapy or combined treatment) are listed in *Table 2*.

Table 2 *The antibiotics used (monotherapy or combined treatment) in this study and number of patients (n) treated with the antibiotic*

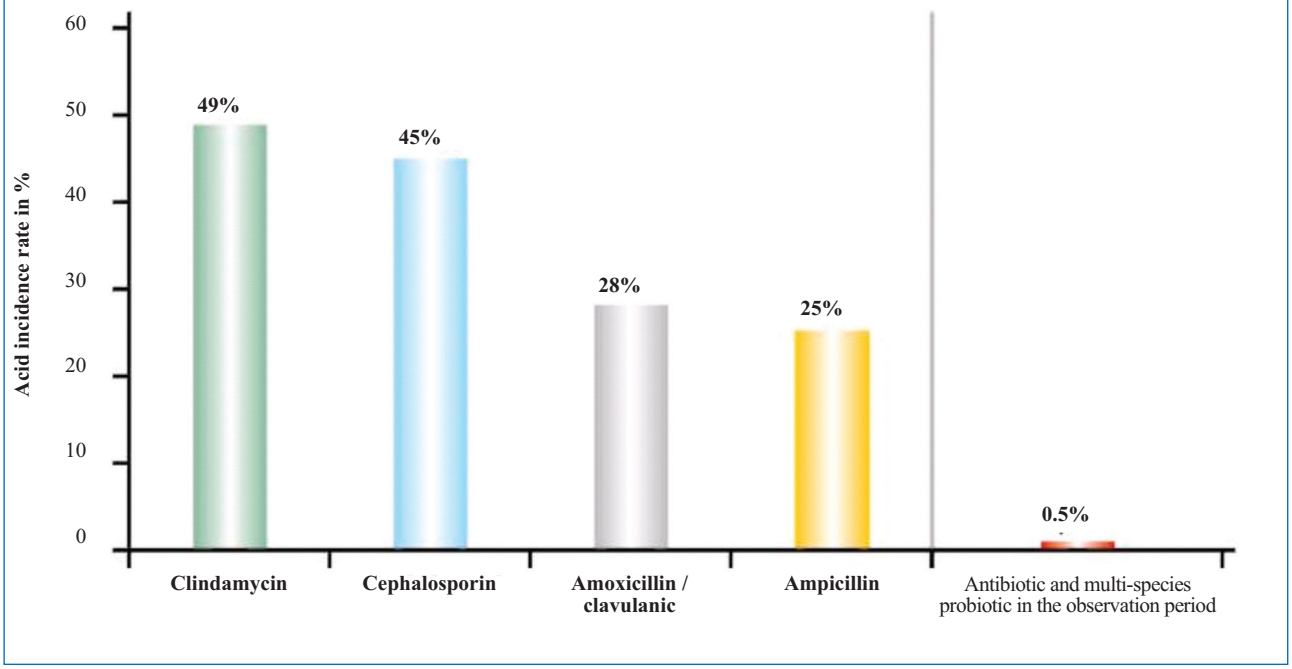
Antibiotic	*n*
Metronidazole	67
Cefuroxime	31
Fosfomycin	(27
Ceftriaxone	(25)
Ampicillin / Sulbactam	(23)
Amoxicillin	(18)
Clarithromycin	(17)
Amoxicillin/ Clavulanic acid	(10)
Vancomycin	(10)
Cefuroxime-axetil	(8)
Piperacillin / Tazobactam	(7)
Rifaximin	(7)
Meropenem	(6)
Penicillin G	(4)
Linezolid	(4)
Clindamycin	(2)
Gentamicin	(1)
Erythromycin	(1)
Norfloxacin	(1)
Refobacina	(1)
Sulfamethoxazole/ Trimethoprim	(1)
Ceftazidime	(1)
Moxifloxaci	(1)

RESULTS

During the 6-month observation period 201 patients were treated with antibiotics. Of these 201 patients, 199 received 5 g of MSP twice daily. Two patients were excluded since it was forgotten to provide them with the MSP. These two patients later developed diarrhoea, and one of them developed pseudomembranous colitis.

Only one out of these 199 patients developed diarrhoea (***Fig. 1***) on the day of discharge from the hospital, which was after 10 days of treatment with penicillin for

Figure 1 *Effect of the multispecies antibiotic on the acid incidence ratio induced by monotherapy or combined treatment with antibioticsof antibiotics 199 Patients were treated (see text for treatment protocol). The data are shown in the last column and are compared with the incidence ratio produced by some commonly used individual antibiotics (data taken from* **6***)*



erysipelas. The patient in question had refused any further treatment and left the hospital. One patient out of 199 developing AAD represents 0.5% of the population, this is much lower than the 5-49% found in literature.

In this study, the administration of MSP:

- showed a significant reduction of diarrhoea and the side effects associated with use of antibiotics (48% against 79%, $p < 0.05$),
- resulted in the stimulation of the mucosal immune system (evidences by the increase of the secretory IgA in the serum)
- increased the levels of those bacteria that were introduced with the product.

Intolerance for MSP was not detected throughout the treatment period. Administration of the compound was not interrupted and side effects were not found. Some of the patients only criticised the taste of the product.

Administration of MSP highly reduced the overall costs for health care because no treatment for AAD or colitis caused by *Clostridium difficile* was required.

CONCLUSIONS

The prevention of AAD by administration of MSP showed highly satisfactory results in this study. The costs of health care could be reduced when the product will be administered during and after antibiotic treatment. Since severe complications such as pseudomembranous colitis can be the result of antibiotic use and could even lead to mortality; prevention of these complications by supplementation of MSP may be suggested.

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