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A mixture of *Escherichia coli* (DSM 17252) and *Enterococcus faecalis* (DSM 16440) for treatment of the irritable bowel syndrome – A randomized controlled trial with primary care physicians

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Abstract *Therapy trials with bacterial compounds in irritable bowel syndrome (IBS) have produced conflicting results. This study was performed in 1988 and 1989, and was re-analysed according to current IBS standards. Two hundred ninety-seven patients with lower abdominal symptoms diagnosed as IBS were treated for 8 weeks by the compound ProSymbioflor® (Symbiopharm GmbH, Herborn, Germany), an autolysate of cells and cell fragments of Enterococcus faecalis and Escherichia coli, or placebo in a double-blinded, randomized fashion. Patients were seen weekly by the physician, who assessed the presence of core IBS symptoms. Responders had at least a 50% decrease in global symptom score (GSS) and in abdominal pain score (APS) reports at ≥1 visit during treatment. The responder rate in GSS to the drug was 102/149 (68.5%) in comparison to placebo with 56/148 (37.8%) ($P < 0.001$), the improvement in APS was 108/149 (72.5%) and 66/148 (44.6%) respectively ($P = 0.001$). The number-needed-to-treat was 3.27 for GSS and 3.59 for the APS report. Kaplan–Meier analysis revealed a mean response time of 4–5 weeks for active treatment and more than 8 weeks for placebo ($P < 0.0001$). Treatment of IBS with the bacterial lysate ProSymbioflor is effective and superior to placebo in reducing typical symptoms of IBS patients seen by general practitioners.*

Keywords *gastroenterology, irritable bowel syndrome, primary care, probiotics.*

INTRODUCTION

Probiotic therapy trials in the irritable bowel syndrome (IBS) have produced conflicting results: 11 studies performed between 1989 and 2005[1] have mainly been negative or have shown minor or insufficient efficacy of different probiotic compounds (lactobacillae, bifidobacteria, inactive *Escherichia coli*, or a mixture thereof), mainly due to small sample sizes, inappropriate control strategies, and/or high placebo response rates and low 'therapeutic gain' of the substances above the placebo response. Only after 2005, randomized and placebo-controlled trials based on current IBS Rome definition and criteria[2–8] have established the value of probiotics in the treatment of IBS, but without being able to identify the mechanisms of action. Efficacy in some clinical gastrointestinal conditions (especially diarrhoea) has been proven[9] but in others, such as inflammatory bowel disease, it is still lacking strong evidence. *In vitro* immunological and *in vivo* functional properties have also been shown for (heat-) inactivated probiotic products,[10,11] and so far only in a small IBS patient population.[12]

Most controlled clinical trials in IBS are currently performed in gastroenterological outpatient settings. It has, however, been noted[13,14] that knowledge and acceptance of these gastroenterological criteria for

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functional bowel disorders such as the Rome criteria for IBS[15,16] are not well developed among primary care physicians. On the other hand, family physicians have established their own criteria for the diagnosis of IBS over the past 20 years, who differ to some degree from those of gastroenterologists: these so-called WONCA criteria, first established in 1983,[17] were developed parallel to the Rome consensus process[18] and were last updated in 1998.[19] These criteria are less restrictive and easier to handle in every-day practice and match the definitions of 'somatoform disorders' (somatic disorders with missing evidence of an organic and a likely psychological origin) as the overall category to which IBS belongs according to the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV[20]).

Currently, no generally approved drugs are available for the treatment of IBS, and new compounds developed during the last decade have shown rather poor efficacy in the range of 10–15% above placebo; placebo response rate, in contrast, have ranged between 40% and 60%.[21] This has resulted in relatively high numbers-needed-to-treat (NNT), e.g. in the range of 10 for tegaserod.[22]

Most drugs in IBS have been tested in specialized gastroenterological centres. However, as most IBS patients are treated in primary care, one may speculate that studies conducted in such a setting may be superior to a GI-conducted study in that more and more representative patients can be included. This is also suggested by a recently published trial of a probiotic bifidobacteria infantis preparation[5] that showed superiority of the drug above placebo at least for a concentration of 10^9 colony-forming units (CFU) compared to placebo; this study was performed in primary care.

The study we report here was conducted in primary care in 1988 and 1989, i.e. after the Manning criteria had been published[23] but before the first version of the Rome criteria for the diagnosis of IBS[24] was available. It was re-analysed according to current standard for IBS clinical trials as set by the US Food and Drug Administration (FDA)[22] and the European Medical Agency (EMEA)[25] to test whether the initially recorded efficacy[26] that had initiated registration of the compound (approved in Switzerland by SWISSMEDIC 4 October 2005 (Reg. No 00675), pending in Germany)[27] would be preserved under these circumstances. The bacterial lysate used here contained 1.5 to 4.5×10^7 CFU mL^{-1} of inactivated cells and cell fragments of *Enterococcus faecalis* (DSM 16440) and *E. coli* (DSM 17252). Both strains have been demonstrated to survive the gastric passage and could be identified in respective stool samples[28] when tested individually. Their mixture and subsequent inactivation is tested for clinical efficacy the first time here.

METHODS

Two hundred ninety-seven patients with the diagnosis of IBS according to the criteria of primary care physicians[19] were recruited in 10 primary care centres between June 1988 and February 1989. The study was conducted according to the national legal requirements (§41(3), Arzneimittelgesetz, as of 20 July 1988), the protocol had been approved by the local ethical committee of the physician organization (Landesärztekammer Hessen, Germany) and patients had given written informed consent to participate.

Initial entry criteria were patients of both genders age 18–70, who had been the treated for IBS symptoms during the last year, who presented with abdominal pain, had a minimum score of 44 points on the Kruis scale[29] and who were willing to absent from any spasmolytics medication during the course of the study. Exclusion criteria were the absence of abdominal pain, the presence of organic origin of symptoms, acute cholecystitis or postcholecystectomy symptoms, acute pancreatic inflammation, a medical history in liver damage, ileus, severe chronic diseases of any kind, acute fever, cachexy, patients who took in spasmolytics during the last 7 days, pregnancy, a Kruis score <44 points, patients who are not fully legal competent and patients evidently unable to cooperate in the trial.

They were assessed during an initial doctor's visit of the patients. Patients identified as having IBS were then randomized to receive either ProSymbioflor (Symbiopharm GmbH, Herborn, Germany) (10 drops = 0.75 mL t.i.d. as an oral liquid during the first week, 20 drops t.i.d. for weeks 2 and 3, and 30 drops t.i.d. thereafter) or placebo, identical in taste and texture in a double-blinded fashion for 8 weeks.

ProSymbioflor is an autolysate of cells and cell fragments of *E. faecalis* (DSM 16440) and *E. coli* (DSM 17252), and 1.5 mL of Prosymbioflor contains 3.0 to 9.0×10^7 CFU of living bacteria at the time of their mixture. After mixing both strains, the compound is immediately exposed to heat and pressure (via autoclave), and is sterile thereafter.

Symptoms were assessed weekly – by interview during a doctor's office visit – to verify improvement or not. The initial evaluation assessed the presence of the core IBS criteria and a number of other symptoms (see Table 1), but was based on the criteria of the International Classification of Health Problems in Primary Care (ICHPPC) of the World Organization of National Colleges, Academies and Academic Associations of

Table 1 Symptoms evaluated that matched or did not match IBS criteria in primary care and in gastroenterology

Symptoms	Primary care ICHPPC-2 (WONCA)*	Gastroenterology	
		Manning	Rome I/II/III
Lower GI pain, spontaneous	Yes	Yes	Yes
Pain, spontaneous, diffuse	Yes	Yes	Yes
Lower GI pain, w/palpation	Yes	No	No
Altered stool consistency	Yes	Yes	Yes
Altered stool frequency	Yes	Yes	Yes
Palpable, tender sigma	Yes	No	No
Bloating	Yes	Yes	Yes
Upper GI pain, spontaneous	Yes	Yes	Yes

*International Classification of Health Problems in Primary Care (ICHPPC) of the World Organization of National Colleges, Academies and Academic Associations of General Practitioners/Family Physicians (WONCA). IBS, irritable bowel syndrome.

General Practitioners/Family Physicians (WONCA)[17,19] and not the gastroenterological set of symptoms proposed by Manning *et al.*[23] or by the first version of the Rome criteria.[24] Responders were identified as those patients who had no IBS symptoms at the end of the trial.

The re-analysis of the data was based on current criteria for IBS and response during clinical trials. Patients had to have abdominal pain, and altered bowel habits (diarrhoea, constipation or both alternating) and bloating from the list of symptoms recorded. From the symptom list and the patients rating of severity (Table 1), a global symptom score (GSS) was computed to match current subjective global assessment criteria.[30] To comply with the EMEA request[25] for two primary endpoints (global assessment and pain), an abdominal pain score (APS) was computed as well. A responder was defined as having at least a 50% improvement during the course of the study,[30] and calculations were performed for GSS and APS separately.

Responders and non-responders were compared for drug efficacy by a number of conventional statistical measures, including Fisher's exact test (chi-square test) for overall efficacy, the NNT and the relative benefit (RB). In addition, a Kaplan–Meier function was calculated for the time needed to achieve 50% improvement in both groups.[31] NNT was calculated as [1/((response rate treatment) – (response rate placebo))] * 100. Relative benefit was calculated as (response rate treatment)/(response rate placebo) * 100. This allows comparison of the efficacy of different drugs in across clinical trials[32] independent of the sample size; e.g. the NNT identifies the number of patients to be treated so that one patient more is responding in the active treatment group than in the placebo group. A small NNT thus indicates that the drug is highly efficient to improve patients' symptoms.

Data analysis is based on the intent-to-treat (ITT) population, with the last value carried forward in case of dropouts. Assuming a placebo responder rate of 50%, 125 patients per group would have been necessary to detect an increase of the responder rate by 20% with an alpha of 0.05 and a power of 0.85. Data analysis was performed in addition for the patients who finished the study according to the protocol [per-protocol (PP) population].

A number of *post hoc* analyses were carried out to estimate the importance of the following factors for the overall assessment of drug efficacy: centre effects, gender effects and symptom duration (less or more than 1 year).

All data are given as mean ± SEM. For all tests, a level of 5% was set to indicate significance.

RESULTS

From the initially seen 312 patients, 297 were recruited while 15 were excluded as non-IBS patients. Eighteen of the 297 patients (each nine in the drug and the placebo arm) dropped out during the course of the study; in five cases – three for active treatment and two for placebo – patients discontinued because of adverse events. In 15 cases, incomplete data were available, so that 264 completed the trial (PP sample) (Fig. 1). Patients in both arms of the study were comparable with respect to basic demographic data (Table 2), and adverse events and reasons for discontinuation were similar between drug and placebo (Tables 3 and 4).

With the exception of two patients in the active treatment group and one patient in the placebo group, all patients reported abdominal pain and diarrhoea or constipation. A differentiation between diarrhoea-predominant and constipation-predominant was not made, but $n = 117$ patients in the medication group (78.5%) and $n = 119$ patients in the placebo group (80.4%) reported alternating symptoms of constipation and diarrhoea.

Gastrointestinal adverse events that occurred during treatment (Table 3) included diarrhoea ($n = 11$ in the medication and $n = 3$ in the placebo group), gastric (upper GI) pain or discomfort ($n = 11$ and $n = 7$ respectively), nausea and vomiting ($n = 2$ and $n = 5$), heartburn ($n = 2$ and $n = 0$), painful bloating ($n = 0$ and $n = 2$), upper and lower abdominal pain ($n = 0$ and $n = 1$), dry mouth ($n = 3$ and $n = 1$) and bleeding

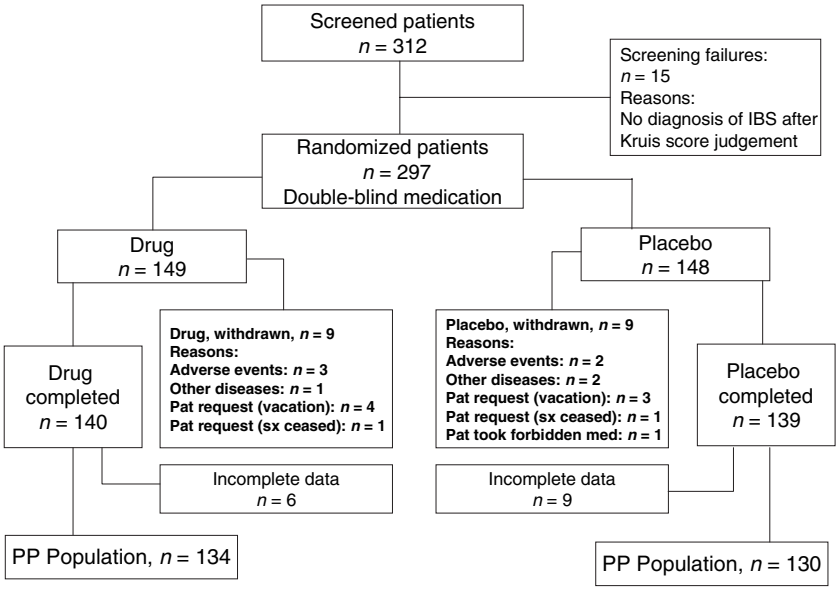

Figure 1 Patient selection and distribution. At the time of study conductance (1988/1989), the number of patients screened was not regularly documented.

Table 2 Demographic data of patients in the placebo and the drug arm of the study

	ProSymbioflor	Placebo
No.	149	148
Females	72	75
Mean age (range) (years)	49.8 (19–70)	49.4 (18–76)
Mean weight (range) (kg)	69.3 (46–100)	68.9 (44–109)
BMI (range) (kg m^{-2})	24.4 (17.2–37.6)	24.0 (17.1–33.2)
Medication during study	–	*N* = 1 (No. 276)

Table 3 Adverse events during the trial in major organ systems in the drug and placebo arm of the study

	Number of adverse events		
Organ class	Placebo	ProSymbioflor	Total
Generalized dysfunctions	2	5	7
Skin responses	4	9	13
Hearing and balance	2	6	8
Psychiatric	6	1	7
Visual system	0	1	1
Digestive system*	20	29	49
Nervous system	1	1	2
Total	35	52	87

*Upper and lower gastrointestinal symptoms: heartburn, nausea and vomiting, bloating, upper and lower abdominal pain or discomfort, diarrhoea and bleeding haemorrhoids.

haemorrhoids (*n* = 1 and *n* = 0). Among these, at least those related to lower GI functions (diarrhoea, lower abdominal pain, bloating) may represent a flare of IBS symptoms rather that true adverse events.

Table 4 Discontinuation of study for adverse events and other reasons

	Number of adverse events		
	Placebo	ProSymbioflor	Total
Adverse events			
Skin responses	1	0	1
Digestive system*	1	3	4
Other reasons			
Other diseases	2	1	3
Patient request (vacation)	4	3	7
Other reasons	1	2	3
Total	9	9	18

*Upper and lower gastrointestinal symptoms: vomiting, diarrhoea and heartburn respectively in the medication group, and abdominal pain in the placebo group.

The gastrointestinal adverse events that led to discontinuation were vomiting, diarrhoea and heartburn respectively in the active medication group, and abdominal pain in the placebo group (Table 4). In only one case (in the placebo group), discontinuation was decided after spasmolytics medication for severe gastrointestinal symptoms (abdominal pain, nausea, vomiting) (Fig. 1).

ITT population

Based on the GSS responder definition, 102/149 (68.5%) were identified as drug responders, while the

response rate in the placebo arm was 56/148 (37.8%) ($P < 0.001$). For APS, the response was 108/149 (72.5%) for the drug and 66/148 (44.6%) for placebo ($P < 0.001$).

The calculated NNT was 3.27 (95% CI: 2.41–5.05) for GSS, and 3.59 (2.59–5.83) for APS. The RB of the drug in comparison to placebo was 1.80 (1.45–2.55) for GSS and 1.62 (1.33–1.97) for APS.

The Kaplan–Maier estimation of the time needed to reach response criteria in both groups were 4–5 weeks in the drug arm and more than 8 weeks in the placebo arm (Kaplan–Meier estimator, $P < 0.0001$) (Fig. 1).

Per-protocol population

In the drug arm, 134 finished treatment per protocol, and in the placebo group, these were 130. The respective efficacy of the drug on GSS was 93/134 (69.4%) and 49/130 (37.7%) for placebo. Abdominal pain score efficacy was 97/137 (72.4%) and 56/130 (43.1%) respectively. This yielded similar NNT and RB for GSS and APS than with the ITT population.

Post hoc analysis

Post hoc analysis revealed no differences in efficacy of the drug between the gender and with shorter duration of the disease. As is evident, patients not meeting (current) IBS criteria, patients with an erythrocyte sedimentation rate >20 mm per 2 h and/or with symptoms of <1 year duration did show high NNT (low RB). While superiority of one centre was noted (NNT: 1.07), all other centres also yielded significant results (NNT: 7.54) (Table 5).

Table 5 *Post hoc* analysis of subgroups of the ITT population with NNT

	Number	Mean NNT	95% CI
IBS conform w/DGVS*	254	2.83	2.13–4.18
IBS not conform w/DGVS	43	38.5	3.1–3.69
ESR ≤20 mm per 2 h	241	2.70	2.05–3.94
ESR >20 mm per 2 h	54	364.00	3.72–3.8
IBS conform w/DGVS and ESR ≤20 mm per 2 h	204	2.27	1.78–3.16
Symptoms ≤1 year	44	2.07	1.47–4.49
Symptoms >1 year	251	3.63	2.53–6.37
Males	117	2.49	1.76–4.22
Females	179	3.98	2.55–9.13
Centre 1	64	1.07	0.98–1.17
All others	233	7.54	3.85–170.9

*DGVS: German Society of Digestive and Metabolic Diseases, that released an IBS consensus statement in 1999.[38] ESR, erythrocyte sedimentation rate; ITT, intent to treat; NNT, number needed to treat, IBS, irritable bowel syndrome.

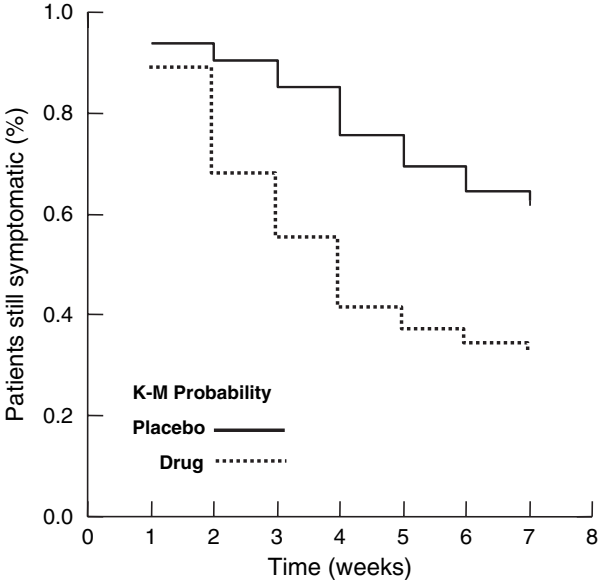

Figure 2 'Survival' plot of drug (dotted line) and placebo group (solid line): the Kaplan–Meier function is calculated for the time (in weeks) needed to reach response criteria (50% symptom improvement). The difference is highly significant ($P < 0.001$).

DISCUSSION

Recently published trials[5–7] have demonstrated overall superiority of probiotic compounds in comparison to placebo in the treatment of IBS, while studies published before 2005 usually were unable to verify this.[1] This was in part due to the fact that small sample sizes usually results in high variability of the placebo response[21] and carry the risk of failure. This is well in line with the large-scale study reported here, which was conducted between June 1988 and February 1989. When analysed according to current standards of IBS definitions[15] and trial requirements,[33] it showed high efficacy of the compound, an inactivated *E. coli* and *E. faecalis* mixture preparation[28] in comparison to placebo.

In vitro immunological and *in vivo* functional properties of inactivated bacterial products have been shown[10,11] also in IBS patients.[12] To our knowledge this is the first double-blinded, randomized and placebo-controlled study in a large population of IBS patients. As different types of inactivation exhibit different functional consequences,[10] and the compound tested here contained both inactivated *E. coli* and Enterococcus cells as well as fragments of bacterial cells, one cannot conclude from the present data on the mechanisms of action, but mediation through the intestinal immune system and direct effects on gut

function seem possible,[11] while direct interactions with the stationary colonic bacteria are rather unlikely.

However, re-analyses such as ours have methodological limitations that need to be acknowledged. One is that data that are required for IBS studies nowadays and that have not been recording previously are limiting the comparability of results to recently analysed and published data. This refers, e.g. to IBS symptom duration and severity that are prerequisites of current IBS studies but are missing in our data set. Others are assessment of symptoms by physicians rather than the currently used 'subjective global assessment' of symptoms and symptom changes by the patient.[30] To compensate for such limitations, the threshold for being a responder was elevated: compared to currently performed trials where patients have to show 'at least 50% symptom improvement',[30] the initial analysis used the criterion 'symptom-free' to define a responder.

The definition of the IBS has substantially changed over the last 2 decades, and the so-called Rome consensus[18] has found its way into academic medicine and gastroenterology, especially with respect to recruitment of IBS patients for clinical trials. Whether it has been accepted in daily routine by gastroenterologists is still a matter of debate,[13] and is has been questioned whether it will even affect the majority of IBS patients that are not seen by a gastroenterologist but consult in primary care.[34,35] Primary care physicians, on the contrary, have developed their own set of criteria defining IBS,[17,19] and frequently do not use the more complex Rome definitions.[14,36] Therefore, the question remains whether or not IBS trials should be conducted in the gastroenterologists or in the primary care physician practice. This may have significant implications, as the gastroenterologist and the Rome criteria rely on the presence of IBS-typical symptoms and potentially add a colonoscopy, while in primary care, the 'tender and palpable colon' but not a colonoscopy is a prerequisite for this diagnosis.

In consequence of this, IBS patients seen in primary care may differ from those seen in the specialized clinic, and may represent a subsample of all IBS patients. The question arises which subset of patients may better be studied in treatment trials, e.g. of new compounds. It may be helpful to study this in direct comparison in a trial setting where IBS patients are recruited by either gastroenterologists or a family physician for the same study design and compound in the future.

While more and more probiotic compounds become available on the market or are seeking approval, their specific mechanisms of action in various clinical conditions remains obscure. Based on the reviewed and meta-analysed data, bifido bacteria,[5–7] lactobacillus[2] and a mixture of different bacterial strains[3,4,8] have been shown to be effective in clinical conditions such as IBS; *E. coli* preparation, in contrast, have so far only been evaluated in non-controlled trials.[1] This casts some doubts on the overall rationale for their use, unless a specific mechanism of action – via the intestinal immune system, the enteric nervous system or otherwise – has been demonstrated to operate. A recent IBS trial[8] that attempted to do this was unable to identify a mechanism (e.g. via short chain fatty acid modulation) but speculated that efficacy must be due to factors other than the presence of induced microbiota itself; this is further supported by the study presented here, as the sterile bacterial lysate that was used in this study may have elicited itsmechanisms of action via direct interaction with the immune system rather than via interaction with the local bacterial colony. However, their low profile for adverse events, their high acceptance in patients and the low NNT as demonstrated here may justify their clinical use, even though the basic scientific knowledge of their mode of operation is still lacking. [37]

REFERENCES

1 Floch MH. Use of diet and probiotic therapy in the irritable bowel syndrome: analysis of the literature. *J Clin Gastroenterol* 2005; **39**: S243–6.

2 O'Mahony L, McCarthy J, Kelly P *et al.* Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005; **128**: 541–51.

3 Kajander K, Hatakka K, Poussa T, Farkkila M, Korpela R. A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6-month intervention. *Aliment Pharmacol Ther* 2005; **22**: 387–94.

4 Kim HJ, Vazquez RoqueMI, Camilleri M *et al.* A randomized controlled trial of a probiotic combination VSL 3 and placebo in irritable bowel syndrome with bloating. *Neurogastroenterol Motil* 2005; **17**: 687–96.

5 Whorwell PJ, Altringer L, Morel J *et al.* Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. *Am J Gastroenterol* 2006; **101**: 1581–90.

6 Fanigliulo L, Comparato G, Aragona G *et al.* Role of gut microflora and probiotic effects in the irritable bowel syndrome. *Acta Biomed* 2006; **77**: 85–9.

7 Guyonnet D, Chassany O, Ducrotte P *et al.* Effect of a fermented milk containing Bifidobacterium animalis DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. *Aliment Pharmacol Ther* 2007; **26**: 475–86.

8 Kajander K, Krogius-Kurikka L, Rinttila T, Karjalainen H, Palva A, Korpela R. Effects of multispecies probiotic supplementation on intestinal microbiota in irritable bowel syndrome. *Aliment Pharmacol Ther* 2007; **26**: 463–73.

9 Allen SJ, Okoko B, Martinez E, Gregorio G, Dans LF. Probiotics for treating infectious diarrhoea. *Cochrane Database Syst Rev* 2004; CD003048.
10 Dehlink E, Domig KJ, Loibichler C *et al.* Heat- and formalin-inactivated probiotic bacteria induce comparable cytokine patterns in intestinal epithelial cell-leucocyte cocultures. *J Food Prot* 2007; **70**: 2417–21.
11 Verdú EF, Bercík P, Bergonzelli GE *et al.* Lactobacillus paracasei normalizes muscle hypercontractility in a murine model of postinfective gut dysfunction. *Gastroenterology* 2004; **127**: 826–37.
12 Tsuchiya J, Barreto R, Okura R, Kawakita S, Fesce E, Marotta F. Single-blind follow-up study on the effectiveness of a symbiotic preparation in irritable bowel syndrome. *Chin J Dig Dis* 2004; **5**: 169–74.
13 Van der Horst HE, van Dülmen AM, Schellevis FG, van Eijk JT, Fennis JF, Bleijenberg G. Do patients with irritable bowel syndrome in primary care really diifer from outpatients with irritable bowel syndrome? *Gut* 1997; **41**: 9969–74.
14 Bijkerk CJ, de Wit NJ, Stalman WA, Knottnerus JA, Hoes AW, Muris JW. Irritable bowel syndrome in primary care: the patients' and doctors' view on symptoms, etiology and management. *Can J Gastroenterol* 2003; **17**: 363–8.
15 Thompson WG, Heaton KW, Irvine EJ, Muller-Lissner SA. Functional bowel disorders and functional abdominal pain. *Gut* 1999; **45**(Suppl. 2): 43–7.
16 Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; **130**: 1480–91.
17 WONCA, Ed. *ICHPPC-2-Defined*, 3rd edn. Oxford: Oxford University Press, 1983.
18 Thompson WG. The road to Rome. *Gastroenterology* 2006; **130**: 1552–6.
19 WONCA, Ed. *ICPC-2, International Classification of Primary Care*, 2nd edn. Oxford: Oxford University Press, 1998.
20 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders (4th edition: Primary Care Version) (DSM-IV-PC)*. Washington, D.C.: APA, 1994.
21 Enck P, Klosterhalfen S. The placebo response in functional bowel disorders: perspectives and putative mechanisms. *Neurogastroenterol Motil* 2005; **17**: 325–31.
22 Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER). *Zelnorm (Tegaserod Maleate) Tablets*, Application no.: 21-200; Approval date: 24 July 2002, Published: 23 October 2002. Available at: http://www.fda.gov/cder/approval/index.htm, accessed on 20 May 2008.
23 Manning AP, Thompson WG, Heaton KW, Morris AF. Towards positive diagnosis of the irritable bowel syndrome. *Br Med J* 1978; **00**: 653–4.
24 Thompson WG. Irritable bowel syndrome: guidelines for the diagnosis. *Gastroenterol Int* 1989; **2**: 92–5.
25 EMEA, Committee for Proprietary Medicinal Products. Points to consider on evaluation of medicinal products for the treatment of irritable bowel syndrome. Document: CMPM/EMW/785/97, Published: 19 March, 2003.
26 Panijel M, Burkard I. Pro-symbioflor for treatment of the irritable colon. *Jatros Naturheilkunde* 1993; **2**(3): 1–4.
27 Wirksamkeit und Verträglichkeit von Pro-Symbioflor®. *Randomisierte, multizentrische, doppelblinde, Placebo-kontrollierte Prüfung an 297 Patienten mit Reizd-armsyndrom unter 8-wöchiger Dauerbehandlung mit Pro-Symbioflor® (Klinische Phase IV)*. Frankfurt: Ergänzender Integrierter Klinischer Studien-Bericht PAZ 9527-5-PS, 24 June, 2005.
28 Rusch V, Hyde RM, Ludley TD. Documentation of identiy, production, harmlessness, efficacy, and durability of Symbioflor preparations. *Mikroökol Ther* 1980; **10**: 173–203.
29 Kruis W, Thieme C, Weinzierl M, Schussler P, Holl J, Paulus W. A diagnostic score for the irritable bowel syndrome Its value in the exclusion of organic disease. *Gastroenterology* 1984; **87**: 1–7.
30 Gordon S, Ameen V, Bagby B, Shahan B, Jhingran P, Carter E. Validation of irritable bowel syndrome Global Improvement Scale: an integrated symptom end point for assessing treatment efficacy. *Dig Dis Sci* 2003; **48**: 1317–23.
31 Petrie A, Sabin C. *Medical Statistics at a Glance*. Oxford: Blackwell, 2007.
32 Altmann DG. Confidence intervals for the number needed to treat. *Br Med J* 1998; **317**: 1309–12.
33 Irvine AJ, Whitehead WE, Chey WD *et al.* Design of treatment trials for functional gastrointestinal disorders. *Gastroenterology* 2006; **130**: 1538–51.
34 Smith GD, Steinke DT, Kinnear M, Penny KI, Pathmanathan N, Penman ID. A comparison of irritable bowel syndrome patients managed in primary and secondary care: the episode IBS study. *Br J Clin Pract* 2004; **54**: 503–7.
35 Yawn BP, Lydick E, Locke GR, Wollan PC, Bertram SL, Kurland MJ. Do published guidelines for evaluation of irritable bowel syndrome reflect practice? *BMC Gastroenterol* 2001; **I**: 11.
36 Saito YA, Talley NJ, Melton LJ II, Fett S, Zinsmeister AR, Locke GR. The effect of new diagnostic criteria for irritable bowel syndrome on community prevalence estimates. *Neurogastroenterol* 2003; **15**: 687–94.
37 Shanahan F. Irritable bowel syndrome: shifting the focus towards the gut microbiota. *Gastroenterology* 2007; **133**: 340–2.
38 Hotz J, Madisch A, Enck P *et al.* The irritable bowel syndrome Definition, diagnosis, pathophysiology and therapy options. *Dtsch Arztebl* 2000; **97**: A3263–70.