BMJ Open

"Effects of a probiotic treatment on symptoms of allergic rhinitis, comparing the probiotic Ent. faecalis to double-blinded placebo, open-label placebo, and to a "no treatment" control - study protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031339
Article Type:	Protocol
Date Submitted by the Author:	29-Apr-2019
Complete List of Authors:	Schaefer, Michael; Medicalschool Berlin, Enck, Paul; University of Tubingen
Keywords:	placebo, allergic rhinitis, probiotics, open-label placebo

Effects of a probiotic treatment on symptoms of allergic rhinitis, comparing the probiotic *Ent. faecalis* to double-blinded placebo, open-label placebo, and to a "no treatment" control - study protocol

Michael Schaefer*[1], Paul Enck [2]

[1] Medical School Berlin, Calandrellistr. 1-9, 12247 Berlin, Germany

[2] University Hospital Tübingen, Dept. of Internal Medicine VI: Psychosomatic Medicine and Psychotherapy, Osianderstraße 5, 72076 Tübingen, Germany

*Correspondence to: **Michael Schaefer, PhD**

Medical School Berlin
Calandrellistr. 1-9
12247 Berlin, Germany
Tel.: +49(0) 30-6117542
Fax: +49(0) 30-6715233
Email: michael.schaefer@medicalschool-berlin.de

Clinical Trial Registration Number:
German Clinical Trials Register DRKS00015804

Short title: Probiotics and placebos in allergic rhinitis

Abstract

Introduction: Allergic rhinitis is very common in the western world. Between 10% and 20% of the general population report symptoms of allergic rhinitis. The main disease-modifying treatment is allergen immunotherapy. In addition, medical treatments are available for symptomatic relief. However, complete symptom resolution is often not achieved. Probiotics have been discussed to be a possible novel treatment for allergic rhinitis. Several studies suggested that gut microbiota may play an important role for allergic diseases, but many of these trials report mixed conclusions. In addition, it is well known that symptoms in allergic rhinitis are prone to exhibit high placebo responses. Moreover, recent studies report that even placebos without deception (open-label placebos, OLP) are highly effective in reducing symptoms of allergic rhinitis.

Methods and Analysis: This study aims to compare the effects seen with a probiotic treatment (*Ent. faecalis*) with effects seen with OLP and with placebo effects seen within a double-blinded placebo provision. Furthermore, we include a "no treatment" condition to examine spontaneous variation of symptoms. The primary outcome is the examination of allergic symptoms. Furthermore, health-related quality of life is examined. This report describes the study design of the randomized controlled trial.

Ethics and dissemination: The study design was approved by the ethical committee of the UKT Department of Psychosomatic Medicine and Psychotherapy, Tübingen, Germany. The trial is registered at the German Clinical Trials Register (www.drks.de, DRKS0001580). The trial results will be published in peer-reviewed journals and at conferences.

Keywords: allergic rhinitis; probiotics; open-label placebos; study protocol

Article Summary

Strengths and limitations of the study

- Probiotic is tested in patients with allergic rhinitis
- Study design included three control arms, two of which involve placebos
- One control-arm is an open-label placebo

Introduction

Allergic diseases are defined as conditions caused by hypersensitivity of the immune system to something in the environment that in general causes no problems in most people. Allergic diseases such as allergic rhinitis affect up to 20 % all people in the developed world (1). Symptoms of allergic rhinitis include, for example, rhinorrhea, pruritus, sneezing, nasal congestion, itching, burning or red eyes, and scratching feelings in the throat. Allergic rhinitis is known to be an IgE-mediated disease (2). The main disease-modifying treatment is allergen immunotherapy, which has been shown to be effective for allergic rhinitis with a high level of evidence (3). Furthermore, medical treatments are available for symptomatic relief.

These medications have been proven to be effective for symptomatic relief, but complete symptom resolution is often not achieved (4). Moreover, drug treatment (e.g. by histamine antagonists) is often associated with severe adverse events such as fatigue that disables or restricts patients to continue their daily activities, e.g. driving cars or working (5). Although last generation histamine antagonists do not show severe adverse events anymore (6, 7), current medications for allergic rhinitis may still have undesirable side effects that affect, for example, quality of life (8, 9).

Recently, it has been suggested that probiotics may be a possible new treatment for allergic rhinitis, in particular, probiotics with a low adverse effects profiles such as lactobacillae and bifidobacteria (10). For example, Watts et al. reported that probiotics had effects on quality of life and reduce medication use in allergic rhinitis (11). Probiotics are (in general) living microorganisms that can be found in foods such as yogurt, sauerkraut, and pickles. Second generation probiotics have been developed as nutritional supplements, to improve their efficacy, sensitivity and specificity in specific

clinical conditions (12). Several studies suggested that gut microbiota may play an important role for immune and allergic diseases.

Effects for probiotics in preventing allergic diseases have been reported in particular when prescribed during the perinatal period (13). When probiotics are administered later, when the allergic disease is already established, studies often report mixed conclusions (e.g., (14-18)). Thus, it remains unclear if probiotics are effective for allergic rhinitis when this disease is well-known for years.

Symptoms of allergic rhinitis vary depending on seasonal changes of allergic load of the environment as well as on individual sensitivity to environmental allergens, for example, due to psychological stress (19). Considering this situation - frequent waxing and waning of symptoms - symptomatic therapies of allergic rhinitis are known to be prone to placebo effects (20-25). While placebo responses may be problematic when testing new therapies, recent studies suggest that the beneficial effects of placebos may be directly used to help patients. We therefore installed three control groups: conventional double-blinded and open-label placebo (OLP) as well as a no-treatment control to adjust for spontaneous symptom variation.

Placebos are defined as composed of inactive ingredients that have no physiological effect on symptoms. Typically, placebos are designed to match active pharmaceuticals in appearance and taste in order to serve as a control condition in double-blind randomized controlled trials. In order to do so, placebos are administered in a concealed way (26). Recent studies (27) now questioned whether such double-blinded provision of placebo is necessary to elicit placebo effects. For example, randomized controlled trials examining the effects of OLP demonstrated significant improvements for patients with Irritable Bowel Syndrome, episodic migraine attacks, chronic lower back pain, depression, and cancer-related fatigue (28-32). In addition, two previous studies showed

that OLPs are highly effective in reducing symptoms of allergic rhinitis (33, 34). A meta-analysis found moderate effects sizes for OLP treatments (35), but also sees some methodological limitations that future studies have to address, e.g., the need for a decepted placebo condition (27, 36).

The objective of this study is to test a probiotic treatment (Ent. faecalis) in patients with allergic rhinitis compared with effects seen by OLP, concealed placebo treatment and no treatment control. The current paper describes the design of this study.

Methods and analysis

The study consists out of four arms. The study arms include a double-blind probiotic / placebo group (group 1 and 2), an open-label placebo group (group 3), and a no-treatment control group (group 4) to control for spontaneous variation of symptoms without treatment. Before and after the treatment we will assess allergic symptoms and health-related quality of life by means of diaries and paper-pencils tests (primary endpoints: Combined symptoms and medication scores, CSMS (37); Rhinitis Quality of Life Questionnaire, RQLQ (38)).

Ethics and Dissemination

The study protocol has been approved by an ethical committee of the University Hospitals, Tübingen, Germany and was registered at the German Clinical Trials Register (www.drks.de, DRKS0001580). All participants will give written informed consent prior to entry to the study and will be made aware that participation is strictly voluntary. Participants may withdraw from the study at any time.

The eventual trial will be published and subsequently disseminated by the university and social media platforms. The results will also be presented at conferences. Study results will be published in a peer-reviewed journal.

Patient and public involvement

Patient and public representatives will be informed about the study (DAAB, Deutscher Allergie und Asthma Bund (German Allergy and Asthma Association)). A summary of the findings will be made available to the DAAB.

Study timeline

The study will be conducted at two sites, the UKT Department of Psychosomatic Medicine and Psychotherapy, Tübingen and Medical School Berlin (MSB), Berlin, Germany.

Recruitment of patients will start before the beginning of the spring allergy season (February 2019). Start of birch pollen season will be marked. The study will be completed in the summer of 2019.

Study duration includes 4 weeks in the treatment phase and another 4 weeks after the end of the experimental phase (open label probiotic phase). Hence, in total the length of the study is 8 weeks. Previous studies reported that similar time periods are effective for this type of probiotic in children with rhinusinusitis (39), and pharmacy recommendations also suggest a minimum of 4 weeks of treatment.

Participants: Inclusion/exclusion criteria

Patients recruitment will use social media, flyers and in particular the website of the DAAB.

We will recruit and include 120 participants with a history of allergic rhinitis for at least two years. An equal fraction of patients of both sexes is intended, but not enforced.

Allergic rhinitis must have been diagnosed by a physician (allergy sub-specialist or general physician). Participants need to show test results of IgE sensitization to aeroallergens (either skin prick test or serum allergen-specific IgE) not older than 24 months prior to the trial in order to assess the severity of allergic rhinitis. We will include participants with seasonal allergic rhinitis only (not perennial allergic rhinitis). Further inclusion criterion is an age between 18-60 years.

Exclusion criteria are a medical history of diabetes, gastrointestinal diseases, use of antibiotic medication in the last 6 weeks, pregnancy, and any known psychiatric or neurological diseases. Furthermore, perennial allergic rhinitis, chronic rhinosinusitis, or any other chronic nasal condition such as anatomical alterations as septum deviation or perforation are excluding criteria. Last, inability to read and understand the study information and insufficient German language skills will exclude from participation in this study.

Study design and interventions

The study design describes a two-center randomized placebo-controlled four-arm study of a nutritional supplement and its effects on symptoms and biomarkers of allergic rhinitis. The probiotic treatment will be compared to two placebo application modes, a conventional double-blinded placebo application and open-label placebo application, and to a no-treatment (untreated group) control arm (see Figure 1). To compensate for randomization, placebo provision, and waiting, all patients will be offered the probiotic for another 4 weeks after the end of the experimental phase (open label probiotic phase).

The probiotic treatment is *Enterococcus faecalis* (DSM 16440), a Gram-positive probiotic species that is constituent of *Symbioflor 1® (*SymbioPharm, Herborn, Germany). It has been demonstrated that Ent. faecalis stably persists in the human gut when orally administered (40). The probiotic is delivered as drops. The placebo

treatment consists of drops containing the carrier solution of the probiotic treatment (lactose-monohydrate, glucose-monohydrate), but will be indistinguishable in color, smell and taste from the probiotic.

Study conductance

After signing the informed consent form patients will fill out baseline questionnaires in order to measure the allergic burden (primary endpoints are CSMS, RQLQ). Subsequently, patients are randomized into one of the four arms of the study.

Patients in the first arm receive the probiotic treatment (as drops) for 4 weeks (N=30). In the second arm, the patients receive placebo drops (N=30) indistinguishable from the probiotic in color and smell/taste. In the third arm the patients receive the same placebo, but are informed that the treatment is a placebo (OLP condition) (N=30). Patients in the fourth arm (N=30) are the no treatment control group; they receive no special therapy but are informed that they are in the untreated group. Patients will be given the supply of the probiotic or placebo, respectively, for 4 weeks (group 1 to 3) and are instructed to ingest 30 drops three times a day (groups 1 to 3), as well as fill out daily diary forms about their allergic symptoms (all groups). All patients are allowed to continue the usual symptomatic medication of their allergic rhinitis (e.g., anti-HR1, nasal corticosteroids etc.). Intake of this symptomatic medication will be used as an endpoint using the CSMS.

Patients will then return to the study center after 4 weeks for a second investigation and questionnaire assessments. At the second visit we will also ask patients to bring their remaining. A blinded research assistance will then check the amount to evaluate compliance. Furthermore, all patients are offered a 4-week supply of the probiotic treatment in an open-label fashion. If they accept, they are asked to provide

questionnaire data on symptoms course over the 4 weeks, but no further site visit in envisioned.

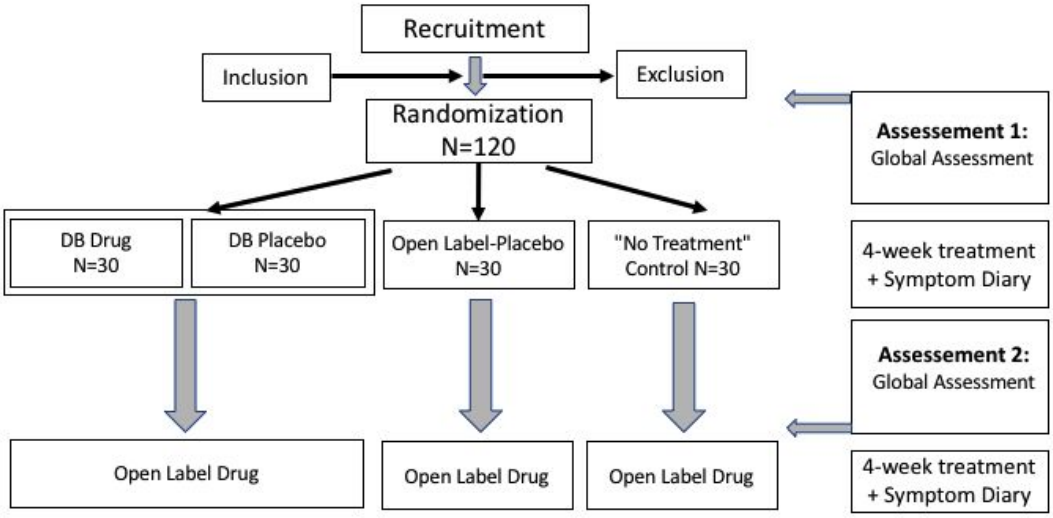


Figure 1: Flow diagram of patient's enrollment.

Measures

Primary endpoint measure is the CSMS, which has been widely used in in previous studies to measure allergic symptoms of allergic rhinitis and is recommend by the European Academy of Allergy and Clinical Immunology (EAACI) (37, 41). The CSMS

measures both symptoms of allergic rhinitis such as nasal and eye symptoms as well as the use of medication. Use of medication will be categorized according the recommendations of the EAACI with respect to H1-antihistaminika, nasal Glucocorticoids, and oral Glucocorticoids (41). Both measures will then build the total symptom score. We will also ask all patients to protocol their allergic burden in a symptom diary on a daily basis during the time of treatment in order to build a daily symptom score based on the CSMS. A second primary endpoint measure is quality of life, measured with the Rhinitis Quality of Life Questionnaire (RQLQ). This questionnaire has 28 questions in seven domains (activity limitations, sleep impairment, non-nasal/eye symptoms, practical problems, nasal symptoms, eye symptoms, and emotional problems) and has strong measurement properties (38).

Secondary endpoint measures will include visual-analogue scales (VAS) to measure the burden of allergic symptoms. VAS have been used to assess the incidence of symptoms or impairment of daily activities (42). Furthermore, we will apply a second questionnaire on quality of life, the SF-36. This questionnaire is a German version of the health survey developed by Ware and Sherbourne (43). This instrument assesses the quality of life with respect to the perception of the health both for patients and healthy people. It includes one multi-item scale that assesses different health concepts such as limitations in physical activities because of health problems, limitations in social activities because of physical or emotional problems, limitations in usual role activities because of physical health problems, bodily pain, general mental health (psychological distress and well-being), limitations in usual role activities because of emotional problems, vitality (energy and fatigue), and general health perceptions. The survey is constructed for self-administration. The SF-36 has also been widely used when measuring effects of allergic rhinitis on everyday life (44).

Analyses

Both primary (CSMS, RQLQ) and secondary endpoints (VAS, SF-36) will be compared between group 1 (probiotic treatment) and group 2 (placebo) for superiority of the probiotic over placebo, between group 2 and group 3 for the size of the placebo effect between open and hidden placebo treatment, and between group 4 to each of the others groups for the contribution of spontaneous variation to the probiotic and placebo effects.

We will calculate adjustments for multiple comparisons (post hoc tests).

Power and sample size

Power calculations of our primary outcome parameter RQLQ were based on previous studies on probiotic effects in allergic rhinitis. Studies on allergic nose symptoms relative to placebo report effect sizes between 0.09 and 2.50 (45-48). Based on these studies we used an estimated effect size of f = 0.6 (CI 1.42 -0.99) to calculate the sample size, resulting in a required number of participants of n = 80. Using analysis of covariance in order to control for baseline scores results in a power at 0.95 to detect a difference in a change from baseline RQLQ, with a 5% level of significance. Given that the difference in change score (means) for this measure is 1.21 and previous studies have shown that mean changes in RQLQ greater than 0.5 can generally be considered as clinically significant, we assume a clinical improvement of the symptoms (38, 49).

In order to account for dropouts, we aim to include 30 patients for each cell.

Blinding

Outcome measurements will be performed by blinded experimenters. Patients in the probiotics and placebo condition will also be blinded, but patients in the OLP condition and in the no-treatment control condition will be aware of this assignment. Effective

blinding in groups 1 and 2 will be ensured by the company that produces and provides the probiotic and placebo (SymbioPharm GmbH, Herborn, Germany); the group assignment list will be withheld until after the final evaluation of the study data.

Discussion

The current article describes the methodology of a trial design on effects of probiotic and OLP on symptoms of allergic rhinitis.

In his hygiene hypothesis Strachan suggested a role of microorganisms for allergic reactions (50). In this theory it is discussed that excessive hygiene may lead to disturbances in the intestinal microbiota. Several studies provide support for this assumption. For example, it has been demonstrated that allergic patients show lower levels of Lactobacillus and Bacteroides (51). Therefore, using probiotic supplementation in allergic rhinitis might be beneficial.

Several studies suggest that probiotics may have an effect on symptoms of allergic rhinitis. For example, Dennis-Wall et al. examined the effects of probiotics in individuals with seasonal allergic conditions and found improvement of rhinoconjunctivitis-specific quality of life (52). Similar effects have been found for a mixture of Bifidobacteria treatment in children with seasonal allergic rhinitis and asthma (53). In addition, animal studies found effects of probiotic treatments on pollen-induced allergic nasal symptoms (54). Nevertheless, the effects of probiotics on allergic rhinitis are still not clear and often inconsistent (in particular when the disease is already established) (14-18).

Furthermore, the mechanisms by which probiotics are thought to be effective in allergic rhinitis are not completely understood. In theory, it is assumed that probiotics exhibit a multitude of mechanisms, ranging for effectively settling is respective mucosal ecological niches (thereby controlling and ousting potentially pathogenic bacteria), via

indicating metabolic effects, to stimulating immunological (anti-inflammatory) responses to novel antigen. For allergic rhinitis, it has been suggested that probiotics may activate or inhibit type 1 T-helper cells by changing the composition of the gut microbiota (52, 55). Probiotics may also stimulate interleukin-10 and thereby inhibiting inflammatory responses (56). Furthermore, probiotics can modify levels of antigen-specific serum IgE levels (57). In addition, Dev et al. found that probiotics suppressed histamine signaling (58). Thus, probiotics might change systemic and adaptive immune response and thereby work as immunomodulators. Furthermore, it has been shown that probiotics may have a dual effect by improving intestinal as well as central nervous system functions (59). Consuming probiotics may lead to a more balanced intestinal flora in allergic rhinitis patients, which might constrain damages due to inflammation. In addition, the more balanced intestinal flora may lead to less severe reactions to allergens. However, further research is needed to fully understand the underlying mechanisms.

Since the effects of probiotics on allergic rhinitis are not clear and often inconsistent (14-18) we here aim to examine effects from a probiotic treatment (Ent. faecalis), compared with two placebo application modes and an untreated group. Ent. faecalis is part of the normal gastrointestinal flora and along with other lactic acid bacteria often used in food products. It has been shown that Ent. faecalis reduces the number and duration of rhinosinusitis episodes in children and adults (39).

The placebo conditions include a conventional double-blinded placebo and an OLP application. The last application was included because recent studies demonstrate that OLP can result in significant effects on various diseases including allergic rhinitis (35).

Although it is well known that symptoms of allergic rhinitis are prone to placebo effects, it is surprising that placebos seem to work even when the patients know that

they receive placebos. In traditional randomized controlled studies placebos are designed to match active pharmaceuticals in order to serve as a control condition. In daily medicine the placebo effect is often is used in a more direct way. For example, a survey of general practioners in Germany reported that 76% administered placebos (60, 61). However, it is considered unethical to prescribe placebos with therapeutic purposes because deception is thought to be necessary and would therefore undermine informed consent and trust (62). Hence, many practioners prescribe "impure" placebos, e.g., doses of medications, which have no intrinsic pharmacological action on patient's symptoms. For example, according to a recent national survey of internists and rheumatologists in the US, only a small number of US physicians used inert placebo pills or injections, but about 50 % gave medications that they think to have no specific effect on patients' conditions (63). Thus, they are prescribed as placebos.

While in the classic understanding it is essential that placebo treatment needs deception of the patient, recent studies report evidence that placebos may work even without concealment or deception. This seems to be very important to profit from beneficial effects of placebos used for a therapeutic purpose in a clear ethical frame. Kaptchuk et al. reported a randomized controlled study showing that patients with IBS symptoms swallowing OLP had higher mean global improvement scores than a control group (27). Similar studies have been reported on different diseases (29-31).

So far it is unclear how OLP exhibits its efficacy. Different mechanisms are discussed and may operate together (35). It has been suggested that the effects of OLP may be described by classical conditioning. Thus, the effects seen by OLP may be explained by a conditioned expectation. In this view, placebos may retrieve a pharmacological memory (64). This is supported by a recent study on pain perception, which showed that an OLP effect exists in patients who had been conditioned for longer, but not for shorter

time periods (65). Embodied cognition is a further way to explain OLP effects. According to this theory mind and world interact via the body and thereby may influence our cognitions (66). In contrast to the previous explanation, no specific conditioning procedure is necessary. In addition, patient-healthcare provider relations may be important when trying to understand the effects of OLP. It is well known that the social interaction of the patient with the healthcare provider may result in feeling socially supported, which may affect the health system.

However, in order to better understand why OLPs may work, it seems important to not only know if OLP may result in similar effect sizes than covert placebos, but also in comparison to other and effective or potentially effective treatment options. Unfortunately, to date there are no OLP studies including also a covert placebo condition, or an effective other therapy option. The current trial design aims to account for this lack of comparison conditions.

Taken together, the present trial aims to test a probiotic treatment (Ent. faecalis) in patients with allergic rhinitis compared with effects seen by OLP, double-blinded placebo treatment, and no treatment control. With the inclusion of these additional control conditions and endpoints we hope to determine the effect of the probiotic treatment as well as OLP on allergic rhinitis.

Competing financial interests statement

MS declares no competing financial interests; PE is a consultant of PrecisionBiotics Inc., Cork, Ireland, as well as of SymbioPharm, Herborn, Germany (the company that provided the probiotic), and Parexel Inc., Durham, NC, USA, companies that produce probiotics. He has also received travel support from Danone, Paris, France, and travel support and speaker honorarium from Biocodex, Paris, France; both companies also produce probiotics.

Funding statement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Authors' contribution

PE and MS both drafted and revised the protocol.

Word count

3388 words

References

1. Wheatley LM, Togias A. Clinical practice. Allergic rhinitis. The New England journal of medicine. 2015;372(5):456-63.
2. Gould HJ, Sutton BJ, Beavil AJ, Beavil RL, McCloskey N, Coker HA, et al. The biology of IGE and the basis of allergic disease. Annual review of immunology. 2003;21:579-628.
3. Calderon MA, Penagos M, Sheikh A, Canonica GW, Durham S. Sublingual immunotherapy for treating allergic conjunctivitis. The Cochrane database of systematic reviews. 2011(7):Cd007685.
4. Schatz M. A survey of the burden of allergic rhinitis in the USA. Allergy. 2007;62 Suppl 85:9-16.
5. Yanai K, Rogala B, Chugh K, Paraskakis E, Pampura AN, Boev R. Safety considerations in the management of allergic diseases: focus on antihistamines. Current medical research and opinion. 2012;28(4):623-42.
6. Novak Z, Yanez A, Kiss I, Kuna P, Tortajada-Girbes M, Valiente R. Safety and tolerability of bilastine 10 mg administered for 12 weeks in children with allergic diseases. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2016;27(5):493-8.
7. Church MK. Efficacy and tolerability of rupatadine at four times the recommended dose against histamine- and platelet-activating factor-induced flare responses and ex vivo platelet aggregation in healthy males. The British journal of dermatology. 2010;163(6):1330-2.
8. Meltzer EO, Gross GN, Katial R, Storms WW. Allergic rhinitis substantially impacts patient quality of life: findings from the Nasal Allergy Survey Assessing Limitations. The Journal of family practice. 2012;61(2 Suppl):S5-10.
9. Meltzer EO, Nathan R, Derebery J, Stang PE, Campbell UB, Yeh WS, et al. Sleep, quality of life, and productivity impact of nasal symptoms in the United States: findings from the Burden of Rhinitis in America survey. Allergy and asthma proceedings. 2009;30(3):244-54.
10. Kim MJ, Ku S, Kim SY, Lee HH, Jin H, Kang S, et al. Safety Evaluations of Bifidobacterium bifidum BGN4 and Bifidobacterium longum BORI. International journal of molecular sciences. 2018;19(5).
11. Watts AM, West NP, Smith PK, Cripps AW, Cox AJ. Probiotics and Allergic Rhinitis: A Simon Two-Stage Design to Determine Effectiveness. Journal of alternative and complementary medicine (New York, NY). 2016;22(12):1007-12.
12. O'Toole PW, Marchesi JR, Hill C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. Nature microbiology. 2017;2:17057.
13. Elazab N, Mendy A, Gasana J, Vieira ER, Quizon A, Forno E. Probiotic administration in early life, atopy, and asthma: a meta-analysis of clinical trials. Pediatrics. 2013;132(3):e666-76.
14. Vliagoftis H, Kouranos VD, Betsi GI, Falagas ME. Probiotics for the treatment of allergic rhinitis and asthma: systematic review of randomized controlled trials. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2008;101(6):570-9.
15. Das RR, Singh M, Shafiq N. Probiotics in treatment of allergic rhinitis. The World Allergy Organization journal. 2010;3(9):239-44.
16. Zajac AE, Adams AS, Turner JH. A systematic review and meta-analysis of probiotics for the treatment of allergic rhinitis. International forum of allergy & rhinology. 2015;5(6):524-32.

17. Guvenc IA, Muluk NB, Mutlu FS, Eski E, Altintoprak N, Oktemer T, et al. Do probiotics have a role in the treatment of allergic rhinitis? A comprehensive systematic review and meta-analysis. American journal of rhinology & allergy. 2016;30(5):157-75.
18. Peng Y, Li A, Yu L, Qin G. The role of probiotics in prevention and treatment for patients with allergic rhinitis: A systematic review. American journal of rhinology & allergy. 2015;29(4):292-8.
19. El Hennawi Del D, Ahmed MR, Farid AM. Psychological stress and its relationship with persistent allergic rhinitis. European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery. 2016;273(4):899-904.
20. Horing B, Weimer K, Muth ER, Enck P. Prediction of placebo responses: a systematic review of the literature. Front Psychol. 2014;5:1079.
21. Elsenbruch S, Enck P. Placebo effects and their determinants in gastrointestinal disorders. Nature reviews Gastroenterology & hepatology. 2015;12(8):472-85.
22. Weimer K, Colloca L, Enck P. Placebo eff ects in psychiatry: mediators and moderators. The lancet Psychiatry. 2015;2(3):246-57.
23. Abramson HA. Psychic factors in allergy and their treatment. Annals of allergy. 1956;14(2):145-51.
24. Czubalski K, Zawisza E. The role of psychic factors in patients with allergic rhinitis. Acta oto-laryngologica. 1976;81(5-6):484-8.
25. del Cuvillo A, Sastre J, Bartra J, Mullol J, DaVila I, Montoro J, et al. Placebo effect in clinical trials involving patients with allergic rhinitis. Journal of investigational allergology & clinical immunology. 2011;21 Suppl 3:40-5.
26. Benedetti F. Placebo effects: from the neurobiological paradigm to translational implications. Neuron. 2014;84(3):623-37.
27. Kaptchuk TJ. Open-Label Placebo: Reflections on a Research Agenda. Perspectives in biology and medicine. 2018;61(3):311-34.
28. Kaptchuk TJ, Friedlander E, Kelley JM, Sanchez MN, Kokkotou E, Singer JP, et al. Placebos without deception: a randomized controlled trial in irritable bowel syndrome. PLoS One. 2010;5(12):e15591.
29. Kam-Hansen S, Jakubowski M, Kelley JM, Kirsch I, Hoaglin DC, Kaptchuk TJ, et al. Altered placebo and drug labeling changes the outcome of episodic migraine attacks. Science translational medicine. 2014;6(218):218ra5.
30. Carvalho C, Caetano JM, Cunha L, Rebouta P, Kaptchuk TJ, Kirsch I. Open-label placebo treatment in chronic low back pain: a randomized controlled trial. Pain. 2016;157(12):2766-72.
31. Kelley JM, Kaptchuk TJ, Cusin C, Lipkin S, Fava M. Open-label placebo for major depressive disorder: a pilot randomized controlled trial. Psychotherapy and psychosomatics. 2012;81(5):312-4.
32. Hoenemeyer TW, Kaptchuk TJ, Mehta TS, Fontaine KR. Open-Label Placebo Treatment for Cancer-Related Fatigue: A Randomized-Controlled Clinical Trial. Sci Rep. 2018;8(1):2784.
33. Schaefer M, Sahin T, Berstecher B. Why do open-label placebos work? A randomized controlled trial of an open-label placebo induction with and without extended information about the placebo effect in allergic rhinitis. PLoS One. 2018;13(3):e0192758.
34. Schaefer M, Harke R, Denke C. Open-Label Placebos Improve Symptoms in Allergic Rhinitis. Psychotherapy and psychosomatics. 2016;85:373-4.
35. Charlesworth JEG, Petkovic, G., Kelley, J.M., Hunter, M., Onakpoya, I., Roberts, N., Miller, F.G., Howick, J. Effects of placebos without deception compared

with no treatment: a systematic review and meta-analysis. J Evid Based Med. 2017;10:97-107.
36. Kaptchuk TJ, Miller FG. Open label placebo: can honestly prescribed placebos evoke meaningful therapeutic benefits? BMJ (Clinical research ed). 2018;363:k3889.
37. Rondon C, Blanca-Lopez N, Campo P, Mayorga C, Jurado-Escobar R, Torres MJ, et al. Specific immunotherapy in local allergic rhinitis: A randomized, double-blind placebo-controlled trial with Phleum pratense subcutaneous allergen immunotherapy. Allergy. 2018;73(4):905-15.
38. Juniper EF, Guyatt GH. Development and testing of a new measure of health status for clinical trials in rhinoconjunctivitis. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1991;21(1):77-83.
39. Kitz R, Martens, U., Zieseniß, E, Enck, P., Rose, M.A. Probiotic E.faecalis – adjuvant therapy
in children with recurrent rhinosinusitis. Central European Journal of Medicine. 2012;7:362-5.
40. Wassenaar TM, Marzorati, M., Beimfohr, C., Siegl, A., Zimmermann, K. Survival of Probiotic E. Coli and Ent. Faecalis in the
Human Host after Oral Intake: Results from in Vitro
and in Vivo Studies. Biotechnology & Microbiology. 2017;2:1-5.
41. Pfaar O, Demoly P, Gerth van Wijk R, Bonini S, Bousquet J, Canonica GW, et al. Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. Allergy. 2014;69(7):854-67.
42. Droessaert V, Timmermans M, Dekimpe E, Seys S, Ceuppens JJ, Fokkens WJ, et al. Real-life study showing better control of allergic rhinitis by immunotherapy than regular pharmacotherapy. Rhinology. 2016;54(3):214-20.
43. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Medical care. 1992;30(6):473-83.
44. Jalali MM, Soleimani R, Alavi Foumani A, Ganjeh Khosravi H. Add-on probiotics in patients with persistent allergic rhinitis: A randomized crossover clinical trial. The Laryngoscope. 2019.
45. Costa DJ, Marteau P, Amouyal M, Poulsen LK, Hamelmann E, Cazaubiel M, et al. Efficacy and safety of the probiotic Lactobacillus paracasei LP-33 in allergic rhinitis: a double-blind, randomized, placebo-controlled trial (GA2LEN Study). European journal of clinical nutrition. 2014;68(5):602-7.
46. Lue KH, Sun HL, Lu KH, Ku MS, Sheu JN, Chan CH, et al. A trial of adding Lactobacillus johnsonii EM1 to levocetirizine for treatment of perennial allergic rhinitis in children aged 7-12 years. International journal of pediatric otorhinolaryngology. 2012;76(7):994-1001.
47. Peng GC, Hsu CH. The efficacy and safety of heat-killed Lactobacillus paracasei for treatment of perennial allergic rhinitis induced by house-dust mite. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2005;16(5):433-8.
48. Wang MF, Lin HC, Wang YY, Hsu CH. Treatment of perennial allergic rhinitis with lactic acid bacteria. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2004;15(2):152-8.
49. Demoly P, Dreyfus I, Dhivert-Donnadieu H, Mesbah K. Desloratadine for the treatment of cypress pollen-induced allergic rhinitis. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2009;103(3):260-6.

50. Strachan DP. Hay fever, hygiene, and household size. BMJ (Clinical research ed). 1989;299(6710):1259-60.
51. Bjorksten B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1999;29(3):342-6.
52. Dennis-Wall JC, Culpepper T, Nieves C, Jr., Rowe CC, Burns AM, Rusch CT, et al. Probiotics (Lactobacillus gasseri KS-13, Bifidobacterium bifidum G9-1, and Bifidobacterium longum MM-2) improve rhinoconjunctivitis-specific quality of life in individuals with seasonal allergies: a double-blind, placebo-controlled, randomized trial. The American journal of clinical nutrition. 2017;105(3):758-67.
53. Miraglia Del Giudice M, Indolfi C, Capasso M, Maiello N, Decimo F, Ciprandi G. Bifidobacterium mixture (B longum BB536, B infantis M-63, B breve M-16V) treatment in children with seasonal allergic rhinitis and intermittent asthma. Italian journal of pediatrics. 2017;43(1):25.
54. Tsunemine S, Isa Y, Ohno H, Hagino S, Yamamura H, Mizutani N, et al. Longitudinal study of effects of oral dosage of Bifidobacterium bifidum G9-1 on Japanese cedar pollen-induced allergic nasal symptoms in guinea pigs. Microbiology and immunology. 2015;59(11):690-9.
55. Lyons A, O'Mahony D, O'Brien F, MacSharry J, Sheil B, Ceddia M, et al. Bacterial strain-specific induction of Foxp3+ T regulatory cells is protective in murine allergy models. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2010;40(5):811-9.
56. Hoyte FCL, Nelson HS. Recent advances in allergic rhinitis. F1000Research. 2018;7.
57. Shida K, Takahashi R, Iwadate E, Takamizawa K, Yasui H, Sato T, et al. Lactobacillus casei strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2002;32(4):563-70.
58. Dev S, Mizuguchi H, Das AK, Matsushita C, Maeyama K, Umehara H, et al. Suppression of histamine signaling by probiotic Lac-B: a possible mechanism of its anti-allergic effect. Journal of pharmacological sciences. 2008;107(2):159-66.
59. Wang H, Lee IS, Braun C, Enck P. Effect of Probiotics on Central Nervous System Functions in Animals and Humans: A Systematic Review. Journal of neurogastroenterology and motility. 2016;22(4):589-605.
60. Linde K, Atmann O, Meissner K, Schneider A, Meister R, Kriston L, et al. How often do general practitioners use placebos and non-specific interventions? Systematic review and meta-analysis of surveys. PLoS One. 2018;13(8):e0202211.
61. Meissner K, Hofner L, Fassler M, Linde K. Widespread use of pure and impure placebo interventions by GPs in Germany. Family practice. 2012;29(1):79-85.
62. Blease CR, Bishop FL, Kaptchuk TJ. Informed consent and clinical trials: where is the placebo effect? BMJ (Clinical research ed). 2017;356:j463.
63. Tilburt JC, Emanuel EJ, Kaptchuk TJ, Curlin FA, Miller FG. Prescribing "placebo treatments": results of national survey of US internists and rheumatologists. BMJ (Clinical research ed). 2008;337:a1938.
64. Colloca L, Howick J. Placebos Without Deception: Outcomes, Mechanisms, and Ethics. International review of neurobiology. 2018;138:219-40.
65. Schafer SM, Colloca L, Wager TD. Conditioned placebo analgesia persists when subjects know they are receiving a placebo. The journal of pain : official journal of the American Pain Society. 2015;16(5):412-20.

66. Fuchs T, Schlimme JE. Embodiment and psychopathology: a phenomenological perspective. Current opinion in psychiatry. 2009;22(6):570-5.

BMJ Open

Effects of a probiotic treatment (Enterococcus faecalis) and open-label placebo on symptoms of allergic rhinitis - study protocol for a randomized controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031339.R1
Article Type:	Protocol
Date Submitted by the Author:	03-Sep-2019
Complete List of Authors:	Schaefer, Michael; Medicalschool Berlin, Enck, Paul; University of Tubingen
Primary Subject Heading:	Immunology (including allergy)
Secondary Subject Heading:	Ethics
Keywords:	placebo, allergic rhinitis, probiotics, open-label placebo

Effects of a probiotic treatment (Enterococcus faecalis) and open-label placebo on symptoms of allergic rhinitis - study protocol for a randomized controlled trial

Michael Schaefer*[1], Paul Enck [2]

[1] Medical School Berlin, Calandrellistr. 1-9, 12247 Berlin, Germany

[2] University Hospital Tübingen, Dept. of Internal Medicine VI: Psychosomatic Medicine and Psychotherapy, Osianderstraße 5, 72076 Tübingen, Germany

*Correspondence to: **Michael Schaefer, PhD**

Medical School Berlin
Calandrellistr. 1-9
12247 Berlin, Germany
Tel.: +49(0) 30-6117542
Fax: +49(0) 30-6715233
Email: michael.schaefer@medicalschool-berlin.de

Clinical Trial Registration Number:
German Clinical Trials Register DRKS00015804

Short title: Probiotics and placebos in allergic rhinitis

Abstract

Introduction: Several studies suggest that gut microbiota may play an important role for allergic diseases. The present trial aims to examine effects of the probiotic Enterococcus faecalis on symptoms of allergic rhinitis in patients. Effects of this probiotic on the immune system have been reported by several studies, but the majority of the previous trials were animal studies. In addition, it is well known that symptoms in allergic rhinitis are prone to exhibit high placebo responses. Moreover, recent studies report that even placebos without deception (open-label placebos) are highly effective in reducing symptoms of allergic rhinitis. Our study design combines both new approaches to assess effects on allergic symptoms in patients. The objective of this study is to compare the effects of a probiotic treatment (Enterococcus faecalis) with effects seen by open-label placebo, concealed placebo treatment and no treatment control. **Methods and Analysis:** A total of 120 patients with allergic rhinitis will be randomly assigned to one of four different groups: a double-blind probiotic / placebo group (groups 1 and 2), an open-label placebo group (group 3), and a no-treatment group (group 4) to control for spontaneous variation of symptoms. The primary outcome is the evaluation of allergic symptoms using the Combined Symptoms Medication Score (CSMS). Furthermore, health-related quality of life is examined (Rhinitis Quality of Life Questionnaire, RQLQ). Secondary outcomes include a visual analogue scale (VAS) on allergic burden and a second quality of life questionnaire (SF-36). This report describes the study design of the randomized controlled trial. **Ethics and dissemination:** The study design was approved by the ethical committee of the UKT Department of Psychosomatic Medicine and Psychotherapy, Tübingen, Germany. The trial is registered at the German Clinical Trials Register (www.drks.de, DRKS0001580). The trial results will be published in peer-reviewed journals and at conferences.

Keywords: allergic rhinitis; probiotics; open-label placebos; study protocol

Article Summary

Strengths and limitations of the study

- This is the first randomized controlled trial designed to assess whether the probiotic treatment Enterococcus faecalis has an effect in patients with seasonal allergic rhinitis (previous studies using Enterococcus faecalis were predominantly animal studies).
- In addition, this study examines the effects of an open-label placebo treatment on symptoms of allergic rhinitis, for the first time comparing effects of an open-label placebo treatment with closed-label (blind) treatment in patients with allergic rhinitis.
- The study design includes three control arms, two of which involve placebos, which allows us to compare the effects of the probiotic with concealed and open placebo conditions and with a no-treatment control.
- A limitation is the length of recruitment in this study (about 6 months), which may effect spontaneous improvement of allergic rhinitis.

Introduction

Allergic diseases are defined as conditions caused by hypersensitivity of the immune system to something in the environment that in general causes no problems in most people. Allergic diseases such as allergic rhinitis affect up to 20 % all people in the developed world (1). Symptoms of allergic rhinitis include, for example, rhinorrhea, pruritus, sneezing, nasal congestion, itching, burning or red eyes, and scratching feelings in the throat. Allergic rhinitis is known to be an IgE-mediated disease (2). The main disease-modifying treatment is allergen immunotherapy, which has been shown to be effective for allergic rhinitis with a high level of evidence (3). Furthermore, medical treatments are available for symptomatic relief.

These medications have been proven to be effective for symptomatic relief, but complete symptom resolution is often not achieved (4). Moreover, drug treatment (e.g. by histamine antagonists) is often associated with severe adverse events such as fatigue that disables or restricts patients to continue their daily activities, e.g. driving cars or working (5). Although last generation histamine antagonists do not show severe adverse events anymore (6, 7), current medications for allergic rhinitis may still have some undesirable side effects (8).

Recently, it has been suggested that probiotics may be a possible new treatment for allergic rhinitis, in particular, probiotics with a low adverse effects profiles such as lactobacillae and bifidobacteria (9). For example, Watts et al. reported that probiotics had effects on quality of life and reduce medication use in allergic rhinitis (10). Probiotics are (in general) living microorganisms that can be found in foods such as yogurt, sauerkraut, and pickles. Several studies suggested that gut microbiota may play an important role for immune and allergic diseases.

Effects for probiotics in preventing allergic diseases have been reported in particular when prescribed during the perinatal period (11). When probiotics are administered later, when the allergic disease is already established, studies often report mixed conclusions (e.g., (12-16)). A recent systematic review and meta-analysis included 22 RCTs. Although there was a high variability among the studies, the results demonstrated significant evidence of beneficial clinical and immunologic effects of probiotics in the treatment of allergic rhinitis (15).

Effects of the probiotic Enterococcus faecalis have been suggested by several studies. For example, it has been demonstrated that Ent. faecalis reduces the number of rhinosinusitis episodes (17). Based on similar studies that report beneficial effects of Ent. faecalis for the immune system (18-23), we hypothesized that this probiotic may also reduce symptoms in seasonal allergic rhinitis.

Symptoms of allergic rhinitis vary depending on seasonal changes of allergic load of the environment as well as on individual sensitivity to environmental allergens. For example, El Hennawi et al. showed improved symptoms of allergic rhinitis when stress is controlled by a pharmacological treatment (24). Considering this situation - frequent waxing and waning of symptoms - symptomatic therapies of allergic rhinitis are known to be prone to placebo effects (25-30). While placebo responses may be problematic when testing new therapies, recent studies suggest that the beneficial effects of placebos may directly be used to help patients. We therefore designed a study with three control groups: conventional double-blind, open-label placebo (OLP) and a no-treatment control to adjust for spontaneous symptom variation.

Placebos are defined as composed of inactive ingredients that have no physiological effects on symptoms. Typically, placebos are designed to match active pharmaceuticals in appearance and taste in order to serve as a control condition in double-blind

randomized controlled trials. In order to do so, placebos are administered in a concealed way (31). Recent studies (32) now questioned whether such double-blinded provision of placebo is necessary to elicit placebo effects. For example, randomized controlled trials examining the effects of OLPs demonstrated significant improvements for patients with irritable bowel syndrome, episodic migraine attacks, chronic lower back pain, depression, and cancer-related fatigue (33-37). In addition, two previous studies showed that OLPs are highly effective in reducing symptoms of allergic rhinitis (38, 39). A meta-analysis found moderate effects sizes for OLP treatments (40), but also sees some methodological limitations that future studies have to address, e.g., the need for a closed-label (blinded) placebo condition (32, 41).

The objective of this study is to test a probiotic treatment (Ent. faecalis) in patients with allergic rhinitis compared with effects seen by OLP, concealed placebo treatment and no treatment control. The current paper describes the design of this study.

Methods and analysis

The study consists of four arms. The study arms include a double-blind probiotic / placebo group (groups 1 and 2), an OLP group (group 3), and a no- treatment control group (group 4) to control for spontaneous variation of symptoms without treatment (see Fig. 1). Before and after the treatment (probiotic/placebo, no treatment) we will assess allergic symptoms and health-related quality of life by means of diaries and paper-pencils tests (primary endpoints: Combined Symptoms and Medication Score, CSMS (42); Rhinitis Quality of Life Questionnaire, RQLQ (43)). The CSMS is a simple and standardized method that balances symptoms and the need for anti-allergic medication. The RQLQ is a disease-specific instrument for evaluating health related quality of life, including patient's physical, social and emotional well-being.

Study setting and timeline

The study will be conducted at two sites, the UKT Department of Psychosomatic Medicine and Psychotherapy, Tübingen and Medical School Berlin (MSB), Berlin, Germany.

Recruitment of patients will start before the beginning of the spring allergy season (February 2019 and 2020). Start of birch pollen season will be marked. The study will be completed in summer of 2020.

Study duration includes 4 weeks in the treatment phase and another 4 weeks after the end of the experimental phase (open label probiotic phase). Hence, in total the length of the study is 8 weeks. Previous studies reported that similar time periods are effective for this type of probiotic in children with rhinosinusitis (17). Pharmacy recommendations also suggest a minimum of 4 weeks of treatment.

Participants: Inclusion/exclusion criteria

Patients recruitment will use social media, flyers and in particular the website of the DAAB (Deutscher Allergie und Asthma Bund (German Allergy and Asthma Association)).

We will recruit and include 120 participants with a history of allergic rhinitis for at least two years. An equal fraction of patients of both sexes is intended, but not enforced. Allergic rhinitis must have been diagnosed by a physician (allergy sub-specialist or general physician). Participants need to show test results of IgE sensitization to aeroallergens (either skin prick test or serum allergen-specific IgE) not older than 24 months prior to the trial.

We will include participants with seasonal allergic rhinitis only (not perennial allergic rhinitis). Further inclusion criterion is an age between 18-60 years.

Exclusion criteria are a medical history of diabetes, gastrointestinal diseases, use of antibiotic medication in the last 6 weeks, pregnancy, and any known psychiatric or neurological diseases. Furthermore, perennial allergic rhinitis, chronic rhinosinusitis, or any other chronic nasal conditions such as anatomical alterations as septum deviation or perforation are excluding criteria. Last, inability to read and understand the study information and insufficient German language skills will exclude from participation in this study.

Study design and interventions

The study design describes a two-center randomized placebo-controlled four-arm study of a probiotic treatment and its effects on symptoms of allergic rhinitis. The probiotic treatment will be compared to two placebo application modes, a conventional double-blind and open-label placebo application, and a no-treatment (untreated group) control arm (see Figure 1). After the end of the experimental phase we will offer the probiotic for another 4 weeks for all patients (open label probiotic phase).

The probiotic treatment is *Enterococcus faecalis* (DSM 16440), a Gram-positive probiotic species that is constituent of *Symbioflor 1® (*SymbioPharm, Herborn, Germany) (cells and autolysate of 1.5 to 4.5 x 10^7 CFU). It has been demonstrated that Ent. faecalis stably persists in the human gut when orally administered (44). The probiotic is delivered as drops. The placebo treatment consists of drops containing the carrier solution of the probiotic treatment (lactose-monohydrate, glucose-monohydrate), but will be indistinguishable in color, smell and taste from the probiotic.

Study conductance

After signing the informed consent form patients will complete baseline questionnaires in order to measure the allergic burden (primary endpoints are CSMS, RQLQ). Subsequently, patients are randomized into one of the four arms of the study.

Patients in the first arm receive the probiotic treatment (as drops) for 4 weeks (N=30). In the second arm, the patients receive placebo drops (N=30) indistinguishable from the probiotic in color and smell/taste. In the third arm the patients receive the same placebo, but are informed that the treatment is a placebo (OLP condition) (N=30). Patients in the fourth arm (N=30) are the no treatment control group; they receive no special therapy but are informed that they are in the untreated group. Patients will be given the supply of the probiotic or placebo, respectively, for 4 weeks (group 1 to 3) and are instructed to ingest 30 drops three times a day (groups 1 to 3), as well as fill out daily diary forms about their allergic symptoms (all groups). All patients are allowed to continue the usual symptomatic medication of their allergic rhinitis (e.g., antihistamines, nasal corticosteroids etc.). Intake of this symptomatic medication will be used as an endpoint using the CSMS.

Patients will then return to the study center after 4 weeks for a second investigation and questionnaire assessments. At the second visit we will also ask patients to bring their remaining. A blinded research assistance will then check the amount to evaluate adherence. Furthermore, all patients are offered a 4-week supply of the probiotic treatment in an open-label fashion. If they accept, they are asked to provide questionnaire data on symptoms course (outcome measures) over the 4 weeks, but no further site visit in envisioned.

Insert Figure 1 about here

Measures

Primary endpoint measure is the CSMS, which has been widely used in in previous studies to measure allergic symptoms of allergic rhinitis and is recommend by the European Academy of Allergy and Clinical Immunology (EAACI) (42, 45). The CSMS measures both symptoms of allergic rhinitis such as nasal and eye symptoms as well as the use of medication. Use of medication will be categorized according the recommendations of the EAACI with respect to antihistamines, nasal Glucocorticoids, and oral Glucocorticoids (45). Both measures will then build the total symptom score. We will also ask all patients to protocol their allergic burden in a symptom diary on a daily basis during the time of treatment in order to build a daily symptom score based on the CSMS. A second primary endpoint measure is quality of life, measured with the Rhinitis Quality of Life Questionnaire (RQLQ). This questionnaire has 28 questions in seven domains (activity limitations, sleep impairment, non-nasal/eye symptoms, practical problems, nasal symptoms, eye symptoms, and emotional problems) and has strong measurement properties (43).

Secondary endpoint measures will include visual-analogue scales (VAS) to measure the burden of allergic symptoms. VAS have been used to assess the incidence of symptoms or impairment of daily activities (46). Furthermore, we will apply a second questionnaire on quality of life, the SF-36. This questionnaire is a German version of the health survey developed by Ware and Sherbourne (47). It assesses the quality of life with respect to the perception of health both for patients and healthy people. The instrument includes one multi-item scale that assesses different health concepts such as limitations in physical activities because of health problems, limitations in social activities because of physical or emotional problems, limitations in usual role activities

because of physical health problems, bodily pain, general mental health (psychological distress and well-being), limitations in usual role activities because of emotional problems, vitality (energy and fatigue), and general health perceptions. The survey is constructed for self-administration. The SF-36 has also been widely used when measuring effects of allergic rhinitis on everyday life (48).

Primary (CSMS, RQLQ) and secondary outcome measures (VAS, SF-36) will be assessed prior the trial, after treatment, and after follow-up.

Adverse events

The safety of patients will be monitored at each study visit. Participants will receive study information containing explicit details on whom to contact in case of an adverse event situation. Furthermore, in this information patients will be told to discontinue the study in an adverse event situation.

Data collection: quality management and storage

Researchers will make appointments for the following dates at the end of the first meeting in order to promote participant retention. Data will be collected in an in-person meeting on paper for each measurement and then electronically recorded at the Medical School Berlin. Once recorded, data will be locked to prevent changes. Missing data because of no-show up will be coded as incomplete. Resulting data is then analyzed with SPSS V25 (IBM Corp., Armonk, NY: USA). All data collected on paper will be marked with a study identification number to prevent identification of the participant and stored in a locked cabinet. Access to the deidentified datasets will be limited to the study authors.

Analyses

Both primary (CSMS, RQLQ) and secondary endpoints (VAS, SF-36) will be compared between group 1 (probiotic treatment) and group 2 (placebo) for superiority of the probiotic over placebo, between group 2 and group 3 for the size of the OLP effect between open and hidden placebo treatment, and between group 4 to each of the others groups for the contribution of spontaneous variation to the probiotic and (open-label) placebo effects.

We will calculate adjustments for multiple comparisons (post hoc tests).

Power and sample size

Power calculations on the effect of probiotics on our primary outcome parameter RQLQ were based on previous studies in allergic rhinitis. Studies on allergic symptoms relative to placebo report effect sizes of d = 0.22 or higher (14, 49, 50). Based on these studies we used an estimated effect size of f = 0.6 (CI 1.42 -0.99) to calculate the sample size, resulting in a required number of participants of n = 80. Using analysis of covariance in order to control for baseline scores results in a power at 0.95 to detect a difference in a change from baseline RQLQ, with a 5% level of significance. Given that the difference in change score (means) for this measure is 1.21 and previous studies have shown that mean changes in RQLQ greater than 0.5 can generally be considered as clinically significant, we assume a clinical improvement of the symptoms (43, 51).

Similar studies investigating the effect of a probiotic mixture (lactobacillus and bifidobacterium) on immune parameters during allergy season calculated that 23 participants per subgroup would be needed to see a difference between probiotic and placebo (52).

Furthermore, based on previous studies we calculated power calculations on the effect of OLPs on symptoms in allergic rhinitis (38, 39). Based on a desired power of .80, an

alpha error probability of .05 and an estimated effect size of f = 0.5, the required number of participants is a priori set to n = 80.

In order to account for dropouts, we aim to include a total of 120 participants.

Blinding and randomization

After completion of first assessments (first visit) group assignment will be determined by opening an opaque envelope (through a research assistant), revealing the participant's randomized assignment to one of the four groups. Randomization is based on a computer-generated random number sequence built by an independent investigator. These researchers will be independent from the members of the study who are responsible for enrolling the participants.

Patients in the probiotics and placebo condition will be blinded (until they finished the study), patients in the OLP condition and in the no-treatment control condition will be aware of this assignment. Effective blinding in groups 1 and 2 will be ensured by the company that produces and provides the probiotic and placebo (SymbioPharm GmbH, Herborn, Germany); the group assignment list will be withheld until the final evaluation of the study data. All outcome measurements will be performed by blinded experimenters.

Patient and public involvement

Patient and public representatives will be informed about the study (DAAB). A summary of the findings will be made available to the DAAB.

Ethics and Dissemination

The study protocol has been approved by an ethical committee of the University Hospital, Tübingen, Germany and was registered at the German Clinical Trials Register

(www.drks.de, DRKS0001580). All participants will give written informed consent prior to entry to the study by a member of the study team and will be made aware that participation is strictly voluntary. Participants may withdraw from the study at any time.

Important protocol modifications will be communicated to the relevant members of the research team.

The eventual trial will be published and subsequently disseminated by the university and social media platforms. The results will also be presented at conferences. Study results will be published in a peer-reviewed journal.

Discussion

The current article describes the methodology of a trial design on effects of a probiotic treatment and OLPs on symptoms of allergic rhinitis.

In his hygiene hypothesis Strachan suggested a role of microorganisms for allergic reactions (53). In this theory it is discussed that excessive hygiene may lead to disturbances in the intestinal microbiota. Numerous studies provide support for this assumption. For example, it has been demonstrated that allergic patients show lower levels of lactobacillus and bacteroides (54). Therefore, using probiotic supplementation in allergic rhinitis might be beneficial.

Several studies suggest that probiotics may have an effect on symptoms of allergic rhinitis. For example, Dennis-Wall et al. examined effects of probiotics in individuals with seasonal allergic conditions and found an improvement of rhinoconjunctivitis-specific quality of life (50). Similar effects have been found for a mixture of bifidobacteria treatment in children with seasonal allergic rhinitis and asthma (55). In addition, animal studies found effects of probiotic treatments on pollen-induced allergic

nasal symptoms (56). Nevertheless, the effects of probiotics on allergic rhinitis are still not clear and often inconsistent (in particular when the disease is already established) (12-16).

Furthermore, the mechanisms by which probiotics are thought to be effective in allergic rhinitis are not fully understood. In theory, it is assumed that probiotics exhibit a multitude of mechanisms, ranging from effectively settling its respective mucosal ecological niches (thereby controlling and ousting potentially pathogenic bacteria), via indicating metabolic effects, to stimulating immunological (anti-inflammatory) responses to novel antigen. For allergic rhinitis, it has been suggested that probiotics may activate or inhibit type 1 T-helper cells by changing the composition of the gut microbiota (50, 57). Probiotics may also stimulate interleukin-10 and thereby inhibiting inflammatory responses (58). Furthermore, probiotics can modify levels of antigen-specific serum IgE levels (59). In addition, Dev et al. found that probiotics suppressed histamine signaling (60). Thus, probiotics might change systemic and adaptive immune response and thereby work as immunomodulators. Furthermore, it has been shown that probiotics may have a dual effect by improving intestinal as well as central nervous system functions (61). Consuming probiotics may lead to a more balanced intestinal flora in allergic rhinitis patients, which might constrain damages due to inflammation. In addition, the more balanced intestinal flora may lead to less severe reactions to allergens. However, further research is needed to fully understand the underlying mechanisms.

Since the effects of probiotics on allergic rhinitis are not clear and often inconsistent (12-16), we here aim to examine effects from a probiotic treatment (Ent. faecalis), compared with two placebo application modes and an untreated group. Ent. faecalis is part of the normal gastrointestinal flora and along with other lactic acid bacteria often

used in food products. Previous studies have already examined Ent. faecalis, but predominantly in animal studies. To our knowledge this is the first RCT that investigates effects of Ent. faecalis in patients with seasonal allergic rhinitis.

Beyond the aim to examine effects of Ent. faecalis on seasonal allergic symptoms in patients, this study has also a second objective, the possible effects of OLPs. The placebo conditions in this study include a conventional double-blinded placebo and an OLP application. The last application was included because recent studies demonstrate that OLP can result in significant effects on various diseases including allergic rhinitis (40).

Although it is well known that symptoms of allergic rhinitis are prone to placebo effects, it is surprising that placebos seem to work even when the patients know that they receive placebos. In traditional randomized controlled studies placebos are designed to match active pharmaceuticals in order to serve as a control condition. In daily medicine the placebo effect is often is used in a more direct way. For example, a survey of general practioners in Germany reported that 76% administered placebos (62, 63). However, it is considered unethical to prescribe placebos with therapeutic purposes because deception is thought to be necessary and would therefore undermine informed consent and trust (64). Hence, many practioners prescribe “impure” placebos, e.g., doses of medications, which have no intrinsic pharmacological action on patient’s symptoms. For example, according to a recent national survey of internists and rheumatologists in the US, only a small number of US physicians used inert placebo pills or injections, but about 50 % gave medications that they think to have no specific effect on patients’ conditions (65). Thus, they are prescribed as placebos.

While in the classic understanding it is essential that placebo treatment needs deception of the patient, recent studies report evidence that placebos may work even without

concealment or deception. This seems to be very important to profit from beneficial effects of placebos used for a therapeutic purpose in a clear ethical frame. Kaptchuk et al. reported a randomized controlled study showing that patients with irritable bowel syndrome symptoms swallowing OLPs had higher mean global improvement scores than a control group (32). Similar studies have been reported on different diseases (34-36).

So far it is unclear how OLP exhibits its efficacy. Different mechanisms are discussed and may operate together (40). It has been suggested that the effects of OLP may be described by classical conditioning. Thus, the effects seen by OLP may be explained by a conditioned expectation. In this view, placebos may retrieve a pharmacological memory (66). This is supported by a recent study on pain perception, which showed that an OLP effect exists in patients who had been conditioned for longer, but not for shorter time periods (67). Embodied cognition is a further way to explain OLP effects. According to this theory mind and world interact via the body and thereby may influence our cognitions (68). In contrast to the previous explanation, no specific conditioning procedure is necessary. In addition, patient-healthcare provider relations may be important when trying to understand the effects of OLP. It is well known that the social interaction of the patient with the healthcare provider may result in feeling socially supported, which may affect the health system.

However, in order to better understand why OLPs may work, it seems important not only to know if OLP may result in similar effect sizes than covert placebos, but also in comparison to other effective or potentially effective treatment options. Unfortunately, to date there are no OLP studies including also a covert placebo condition, or an effective other therapy option. The current trial design aims to account for this lack of comparison conditions.

Taken together, the present trial aims to test a probiotic treatment (Ent. faecalis) in patients with allergic rhinitis compared with effects seen by OLP, double-blinded placebo treatment, and no treatment control. With the inclusion of these additional control conditions and endpoints we hope to determine the effect of the probiotic treatment as well as OLPs on allergic rhinitis.

Acknowledgements

We used the SPIRIT checklist when writing this report.

Competing financial interests statement

MS declares no competing financial interests; PE is a consultant of PrecisionBiotics Inc., Cork, Ireland, as well as of SymbioPharm, Herborn, Germany (the company that provided the probiotic), and Parexel Inc., Durham, NC, USA, companies that produce probiotics. He has also received travel support from Danone, Paris, France, and travel support and speaker honorarium from Biocodex, Paris, France; both companies also produce probiotics.

Funding statement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Authors' contribution

PE and MS both drafted and revised the protocol.

Word count

3905 words

References

1. Wheatley LM, Togias A. Clinical practice. Allergic rhinitis. The New England journal of medicine. 2015;372(5):456-63.
2. Gould HJ, Sutton BJ, Beavil AJ, Beavil RL, McCloskey N, Coker HA, et al. The biology of IGE and the basis of allergic disease. Annual review of immunology. 2003;21:579-628.
3. Calderon MA, Penagos M, Sheikh A, Canonica GW, Durham S. Sublingual immunotherapy for treating allergic conjunctivitis. The Cochrane database of systematic reviews. 2011(7):Cd007685.
4. Schatz M. A survey of the burden of allergic rhinitis in the USA. Allergy. 2007;62 Suppl 85:9-16.
5. Yanai K, Rogala B, Chugh K, Paraskakis E, Pampura AN, Boev R. Safety considerations in the management of allergic diseases: focus on antihistamines. Current medical research and opinion. 2012;28(4):623-42.
6. Novak Z, Yanez A, Kiss I, Kuna P, Tortajada-Girbes M, Valiente R. Safety and tolerability of bilastine 10 mg administered for 12 weeks in children with allergic diseases. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2016;27(5):493-8.
7. Church MK. Efficacy and tolerability of rupatadine at four times the recommended dose against histamine- and platelet-activating factor-induced flare responses and ex vivo platelet aggregation in healthy males. The British journal of dermatology. 2010;163(6):1330-2.
8. Horak F, Stubner UP. Comparative tolerability of second generation antihistamines. Drug safety. 1999;20(5):385-401.
9. Kim MJ, Ku S, Kim SY, Lee HH, Jin H, Kang S, et al. Safety Evaluations of Bifidobacterium bifidum BGN4 and Bifidobacterium longum BORI. International journal of molecular sciences. 2018;19(5).
10. Watts AM, West NP, Smith PK, Cripps AW, Cox AJ. Probiotics and Allergic Rhinitis: A Simon Two-Stage Design to Determine Effectiveness. Journal of alternative and complementary medicine (New York, NY). 2016;22(12):1007-12.
11. Elazab N, Mendy A, Gasana J, Vieira ER, Quizon A, Forno E. Probiotic administration in early life, atopy, and asthma: a meta-analysis of clinical trials. Pediatrics. 2013;132(3):e666-76.
12. Vliagoftis H, Kouranos VD, Betsi GI, Falagas ME. Probiotics for the treatment of allergic rhinitis and asthma: systematic review of randomized controlled trials. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2008;101(6):570-9.
13. Das RR, Singh M, Shafiq N. Probiotics in treatment of allergic rhinitis. The World Allergy Organization journal. 2010;3(9):239-44.
14. Zajac AE, Adams AS, Turner JH. A systematic review and meta-analysis of probiotics for the treatment of allergic rhinitis. International forum of allergy & rhinology. 2015;5(6):524-32.
15. Guvenc IA, Muluk NB, Mutlu FS, Eski E, Altintoprak N, Oktemer T, et al. Do probiotics have a role in the treatment of allergic rhinitis? A comprehensive systematic review and meta-analysis. American journal of rhinology & allergy. 2016;30(5):157-75.
16. Peng Y, Li A, Yu L, Qin G. The role of probiotics in prevention and treatment for patients with allergic rhinitis: A systematic review. American journal of rhinology & allergy. 2015;29(4):292-8.
17. Kitz R, Martens, U., Zieseniß, E, Enck, P., Rose, M.A. Probiotic E.faecalis – adjuvant therapy

in children with recurrent rhinosinusitis. Central European Journal of Medicine. 2012;7:362-5.
18. Castro MS, Molina, M.A., Azpiroz, M.B., Diaz, A.M., Ponzio, R., Sparo, M.D., Manghi, M.A., Canellada, A.M. Probiotic activity of Enterococcus faecalis CECT7121: effects on mucosal immunity and intestinal epithelial cells. J Appl Microbiol. 2016;121:1117-29.
19. Castro MS, Azpiroz MB, Molina MA, Mourelle AC, Alaniz FS, Maldonado AM, et al. Preliminary studies on the prevention of the ovalbumin-induced allergic response by Enterococcus faecalis CECT7121 in mice. International archives of allergy and immunology. 2012;157(1):11-20.
20. Zhu L, Shimada T, Chen R, Lu M, Zhang Q, Lu W, et al. Effects of lysed Enterococcus faecalis FK-23 on experimental allergic rhinitis in a murine model. Journal of biomedical research. 2012;26(3):226-34.
21. Zhu LP, Zhang QZ, Shimada T, Enomoto T, Cheng L. [Anti-allergic effects of the probiotic preparations of enterococcus on experimental allergic rhinitis in mice]. Zhonghua er bi yan hou tou jing wai ke za zhi = Chinese journal of otorhinolaryngology head and neck surgery. 2013;48(7):555-62.
22. Shimada T, Cheng L, Shi HB, Hayashi A, Motonaga C, Tang J, et al. Effect of lysed Enterococcus faecalis FK-23 on allergen-induced immune responses and intestinal microflora in antibiotic-treated weaning mice. Journal of investigational allergology & clinical immunology. 2007;17(2):70-6.
23. Shimada T, Zhu LP, Yin M, Motonaga C, Li HB, Shi HB, et al. Effects of lysed Enterococcus faecalis FK-23 on allergen-induced peritoneal accumulation of eosinophils and serum total IgE concentration in inbred mice. Asian Pacific journal of allergy and immunology. 2008;26(2-3):137-41.
24. El Hennawi Del D, Ahmed MR, Farid AM. Psychological stress and its relationship with persistent allergic rhinitis. European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery. 2016;273(4):899-904.
25. Horing B, Weimer K, Muth ER, Enck P. Prediction of placebo responses: a systematic review of the literature. Front Psychol. 2014;5:1079.
26. Elsenbruch S, Enck P. Placebo effects and their determinants in gastrointestinal disorders. Nature reviews Gastroenterology & hepatology. 2015;12(8):472-85.
27. Weimer K, Colloca L, Enck P. Placebo eff ects in psychiatry: mediators and moderators. The lancet Psychiatry. 2015;2(3):246-57.
28. Abramson HA. Psychic factors in allergy and their treatment. Annals of allergy. 1956;14(2):145-51.
29. Czubalski K, Zawisza E. The role of psychic factors in patients with allergic rhinitis. Acta oto-laryngologica. 1976;81(5-6):484-8.
30. del Cuvillo A, Sastre J, Bartra J, Mullol J, DaVila I, Montoro J, et al. Placebo effect in clinical trials involving patients with allergic rhinitis. Journal of investigational allergology & clinical immunology. 2011;21 Suppl 3:40-5.
31. Benedetti F. Placebo effects: from the neurobiological paradigm to translational implications. Neuron. 2014;84(3):623-37.
32. Kaptchuk TJ. Open-Label Placebo: Reflections on a Research Agenda. Perspectives in biology and medicine. 2018;61(3):311-34.
33. Kaptchuk TJ, Friedlander E, Kelley JM, Sanchez MN, Kokkotou E, Singer JP, et al. Placebos without deception: a randomized controlled trial in irritable bowel syndrome. PLoS One. 2010;5(12):e15591.

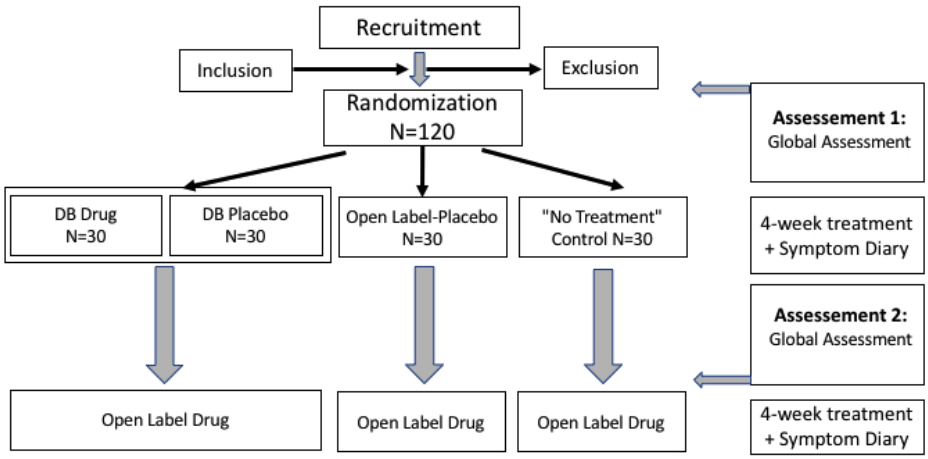
34. Kam-Hansen S, Jakubowski M, Kelley JM, Kirsch I, Hoaglin DC, Kaptchuk TJ, et al. Altered placebo and drug labeling changes the outcome of episodic migraine attacks. Science translational medicine. 2014;6(218):218ra5.
35. Carvalho C, Caetano JM, Cunha L, Rebouta P, Kaptchuk TJ, Kirsch I. Open-label placebo treatment in chronic low back pain: a randomized controlled trial. Pain. 2016;157(12):2766-72.
36. Kelley JM, Kaptchuk TJ, Cusin C, Lipkin S, Fava M. Open-label placebo for major depressive disorder: a pilot randomized controlled trial. Psychotherapy and psychosomatics. 2012;81(5):312-4.
37. Hoenemeyer TW, Kaptchuk TJ, Mehta TS, Fontaine KR. Open-Label Placebo Treatment for Cancer-Related Fatigue: A Randomized-Controlled Clinical Trial. Sci Rep. 2018;8(1):2784.
38. Schaefer M, Sahin T, Berstecher B. Why do open-label placebos work? A randomized controlled trial of an open-label placebo induction with and without extended information about the placebo effect in allergic rhinitis. PLoS One. 2018;13(3):e0192758.
39. Schaefer M, Harke R, Denke C. Open-Label Placebos Improve Symptoms in Allergic Rhinitis. Psychotherapy and psychosomatics. 2016;85:373-4.
40. Charlesworth JEG, Petkovic, G., Kelley, J.M., Hunter, M., Onakpoya, I., Roberts, N., Miller, F.G., Howick, J. Effects of placebos without deception compared with no treatment: a systematic review and meta-analysis. J Evid Based Med. 2017;10:97-107.
41. Kaptchuk TJ, Miller FG. Open label placebo: can honestly prescribed placebos evoke meaningful therapeutic benefits? BMJ (Clinical research ed). 2018;363:k3889.
42. Rondon C, Blanca-Lopez N, Campo P, Mayorga C, Jurado-Escobar R, Torres MJ, et al. Specific immunotherapy in local allergic rhinitis: A randomized, double-blind placebo-controlled trial with Phleum pratense subcutaneous allergen immunotherapy. Allergy. 2018;73(4):905-15.
43. Juniper EF, Guyatt GH. Development and testing of a new measure of health status for clinical trials in rhinoconjunctivitis. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1991;21(1):77-83.
44. Wassenaar TM, Marzorati, M., Beimfohr, C., Siegl, A., Zimmermann, K. Survival of Probiotic E. Coli and Ent. Faecalis in the Human Host after Oral Intake: Results from in Vitro and in Vivo Studies. Biotechnology & Microbiology. 2017;2:1-5.
45. Pfaar O, Demoly P, Gerth van Wijk R, Bonini S, Bousquet J, Canonica GW, et al. Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. Allergy. 2014;69(7):854-67.
46. Droessaert V, Timmermans M, Dekimpe E, Seys S, Ceuppens JJ, Fokkens WJ, et al. Real-life study showing better control of allergic rhinitis by immunotherapy than regular pharmacotherapy. Rhinology. 2016;54(3):214-20.
47. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Medical care. 1992;30(6):473-83.
48. Jalali MM, Soleimani R, Alavi Foumani A, Ganjeh Khosravi H. Add-on probiotics in patients with persistent allergic rhinitis: A randomized crossover clinical trial. The Laryngoscope. 2019.
49. Costa DJ, Marteau P, Amouyal M, Poulsen LK, Hamelmann E, Cazaubiel M, et al. Efficacy and safety of the probiotic Lactobacillus paracasei LP-33 in allergic rhinitis: a double-blind, randomized, placebo-controlled trial (GA2LEN Study). European journal of clinical nutrition. 2014;68(5):602-7.

50. Dennis-Wall JC, Culpepper T, Nieves C, Jr., Rowe CC, Burns AM, Rusch CT, et al. Probiotics (Lactobacillus gasseri KS-13, Bifidobacterium bifidum G9-1, and Bifidobacterium longum MM-2) improve rhinoconjunctivitis-specific quality of life in individuals with seasonal allergies: a double-blind, placebo-controlled, randomized trial. The American journal of clinical nutrition. 2017;105(3):758-67.
51. Demoly P, Dreyfus I, Dhivert-Donnadieu H, Mesbah K. Desloratadine for the treatment of cypress pollen-induced allergic rhinitis. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2009;103(3):260-6.
52. Koyama T, Kirjavainen, P.V., Fisher, C., Anukam, K., Summers, K., Hekmat, S., Reid, G. Development and pilot evaluation of a novel probiotic mixture for the management of seasonal allergic rhinitis. Can J Microbiol. 2010;56:730-8.
53. Strachan DP. Hay fever, hygiene, and household size. BMJ (Clinical research ed). 1989;299(6710):1259-60.
54. Bjorksten B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1999;29(3):342-6.
55. Miraglia Del Giudice M, Indolfi C, Capasso M, Maiello N, Decimo F, Ciprandi G. Bifidobacterium mixture (B longum BB536, B infantis M-63, B breve M-16V) treatment in children with seasonal allergic rhinitis and intermittent asthma. Italian journal of pediatrics. 2017;43(1):25.
56. Tsunemine S, Isa Y, Ohno H, Hagino S, Yamamura H, Mizutani N, et al. Longitudinal study of effects of oral dosage of Bifidobacterium bifidum G9-1 on Japanese cedar pollen-induced allergic nasal symptoms in guinea pigs. Microbiology and immunology. 2015;59(11):690-9.
57. Lyons A, O'Mahony D, O'Brien F, MacSharry J, Sheil B, Ceddia M, et al. Bacterial strain-specific induction of Foxp3+ T regulatory cells is protective in murine allergy models. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2010;40(5):811-9.
58. Hoyte FCL, Nelson HS. Recent advances in allergic rhinitis. F1000Research. 2018;7.
59. Shida K, Takahashi R, Iwadate E, Takamizawa K, Yasui H, Sato T, et al. Lactobacillus casei strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2002;32(4):563-70.
60. Dev S, Mizuguchi H, Das AK, Matsushita C, Maeyama K, Umehara H, et al. Suppression of histamine signaling by probiotic Lac-B: a possible mechanism of its anti-allergic effect. Journal of pharmacological sciences. 2008;107(2):159-66.
61. Wang H, Lee IS, Braun C, Enck P. Effect of Probiotics on Central Nervous System Functions in Animals and Humans: A Systematic Review. Journal of neurogastroenterology and motility. 2016;22(4):589-605.
62. Linde K, Atmann O, Meissner K, Schneider A, Meister R, Kriston L, et al. How often do general practitioners use placebos and non-specific interventions? Systematic review and meta-analysis of surveys. PLoS One. 2018;13(8):e0202211.
63. Meissner K, Hofner L, Fassler M, Linde K. Widespread use of pure and impure placebo interventions by GPs in Germany. Family practice. 2012;29(1):79-85.
64. Blease CR, Bishop FL, Kaptchuk TJ. Informed consent and clinical trials: where is the placebo effect? BMJ (Clinical research ed). 2017;356:j463.

65. Tilburt JC, Emanuel EJ, Kaptchuk TJ, Curlin FA, Miller FG. Prescribing "placebo treatments": results of national survey of US internists and rheumatologists. BMJ (Clinical research ed). 2008;337:a1938.
66. Colloca L, Howick J. Placebos Without Deception: Outcomes, Mechanisms, and Ethics. International review of neurobiology. 2018;138:219-40.
67. Schafer SM, Colloca L, Wager TD. Conditioned placebo analgesia persists when subjects know they are receiving a placebo. The journal of pain : official journal of the American Pain Society. 2015;16(5):412-20.
68. Fuchs T, Schlimme JE. Embodiment and psychopathology: a phenomenological perspective. Current opinion in psychiatry. 2009;22(6):570-5.

Figure legends

Figure 1: Flow diagram of patient's enrollment.



254x142mm (72 x 72 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	1 (see DRK)
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,20
	5b	Name and contact information for the trial sponsor	n.a.
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	n.a.
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n.a.

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7
	6b	Explanation for choice of comparators	7
Objectives	7	Specific objectives or hypotheses	7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n.a.
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8, Fig. 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	14
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	14
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	14
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	14
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	14
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11,12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	12

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n.a.
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	12
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n.a.
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n.a.
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	14
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	15

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	15
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n.a.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12,13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n.a.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14,15
	31b	Authorship eligibility guidelines and any intended use of professional writers	n.a.
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	14
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	see appendix
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n.a.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.