PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effects of a probiotic treatment (Enterococcus faecalis) and open-label placebo on symptoms of allergic rhinitis - study protocol for a randomized controlled trial
AUTHORS	Schaefer, Michael; Enck, Paul

VERSION 1 – REVIEW

REVIEWER	Ibon Eguiluz & Mohamed Shamji Allergy and Clinical Immunology. Immune Regulation and Tolerance. Imperial College. London, UK.
REVIEW RETURNED	10-Dec-2018

GENERAL COMMENTS	The authors present a study protocol for assessing the effectiveness of a probiotic to treat allergic rhinitis, as compared to double-blinded and open placebo, and to a untreated groups. With this study, the authors aim to investigate two relevant aspects: 1/the potential of probiotics in allergic rhinitis; and 2/the extent of different placebo effects, and the potential of open placebo for the management of allergic rhinitis. This two research questions are timely and relevant. Nevertheless, the planned study displays several methodological limitations, which will make difficult to infer valid conclusions from the results. Main points: General Comments: 1.-The two objectives of the study are very different an unrelated. It would be desirable to focus only on the effect of probiotics or on the potential of open placebo treatment for allergic rhinitis. 2.-There is a general confusion throughout the manuscript between allergic rhinitis and chronic rhinosinusitis. These are two different diseases, with different immunopathology and management. The authors probably want to focus on allergic rhinitis, but they refer to “allergic rhinosinusitis (ARS)” throughout the text. This term is not used in the published literature on the topic. Allergic rhinitis patients may have sinusitis episodes as consequence of their nasal inflammation, but the sinusitis in this scenario is a complication rather than a component of the underlying disease. 3.-The authors do not mention in any section the main disease-modifying treatment for allergic rhinitis, which is allergen immunotherapy. This treatment has proven effective for allergic rhinitis, with a level of evidence way stronger than that of probiotics (Calderon, Cochrane 2011). Introduction: 4.-It is not mentioned that allergic rhinitis is an IgE-mediated disease.

	5.-Page 5, Line 23: last generation anti-HR1 drugs do not induce severe adverse effect like first generation anti-HR1 (Novak, PAI 2016; Church, Br J Dermatol 2010) 6.-Page 5, line 27: several studies and metanalysis suggest that probiotics might be effective in preventing allergic diseases if administered during the perinatal period (Elazab, Pediatrics 2013), whereas their therapeutic effect once the disease is established is more limited. Other studies, as indicated by the authors, show therapeutic potential. This controversy should be addressed in the introduction, as a mean to justify the goals of the study. 7.-Page 5, 51: please, elaborate what the author mean with "individual sensitivity to environmental allergens". Do the author have a reference to support this statement? 8.-Page 5, lines 59-60: this sentence needs a supportive reference. Methods and analysis: 9.-Page 6, line 56-57: the specific score to measure symptoms of allergic rhinitis should be clarified from the beginning of the methodology section. There are several systems the authors may choose to use (visual analogue scale, Lebel score, total symptom and medication score, among others). 10.-Page 7, line 3: the authors intend to infer the severity of rhinitis symptoms from the changes in the nasal microbiota. Yet a reference is provided, this approach is quite controversial. For the sake of simplicity, it would be desirable to use the measurement of the nasal microbiota just as an endpoint of the study. Please, explain already how the microbiota will be measured. 11.- Page 7, line 3-4: using MRI to assess the volume of the nasal cavity will make the study less comparable with other investigations of therapies for allergic rhinitis. Moreover, the authors do not intend to perform a nasal allergen provocation before and after the treatment period. This aspect is a clear limitation of the study, as the current design will not allow differentiate between the nasal inflammation caused by allergens, or by any other trigger (e.g. viral infection). Combining a nasal allergen provocation with one of the standards methods to measure nasal patency (acoustic rhinometry, rhinomanometry) for assessing the response to the intervention would add a lot to the study (Auge, Allergy 2018, Pfaar, Allergy 2018). Moreover, with the current methodology, the treatment period needs to occur simultaneously than the allergen season in order to make sure the patients are exposed to the allergen. This aspect is another limitation, which will create several logistical difficulties. 12.- Page 7, line 23-26: do the authors plan to publish the result in a peer-review journal? 13.- Page 8, line 3-4: Is 4 week a period long enough for the probiotics to induce significant changes? Probiotics are supposed to promote immunoregulatory pathways with the generation of different types of regulatory immune cells (Marsland, Nat Rev Immunol 2014). Please, elaborate and cite articles to justify why this duration is appropriate. 14.-Page 8, line 21-28: it is not clear if the diagnosis of allergic rhinitis will be confirmed. Do the patients need to have a confirmation of IgE sensitization to aeroallergens (skin prick test or serum allergen-specific IgE)? Do the patients need to be diagnosed by an Allergy sub-specialist, or by any physician? Do the authors intend to include only individuals with seasonal allergic rhinitis, or also with perennial forms of the disease? Please, specify.

	15.-Page 8, line 31-40: chronic rhinosinusitis, or any other chronic nasal condition (severe anatomical alterations as septum deviation or perforation) should be considered as exclusion criteria. 16.-Page 9, line 5-6: please, indicate that these observations are based on mice studies. 17.-Page 9, line 27-28 line 58-59: “nasal mucosal samples”, the current wording suggests the authors are taking a mucosal biopsy. 18.-Page 9, line 52-54: a thorough explanation of which medications will be allowed is needed. Are the authors talking about anti-HR1, nasal corticosteroids, decongestant sprays, or others? How do the authors intend to normalize the intake of symptomatic treatment among the different study groups? This aspect could greatly affect the validity of the results. Alternatively, the study could use the intake of symptomatic medication as an endpoint (e.g. use the combined symptoms and medication score, Rondon, Allergy 2018). Please, replace “sinusitis” by “rhinitis”. 19.-Page 11, line 1-19: the symptom score the authors intend to use does not seem appropriate, as it grades symptoms related to many other allergic and non-allergic diseases (skin and intestinal conditions) besides allergic rhinitis. This score is not appropriate for the goals and scope of this study. 20.-Page 11, line 58-60: It is not clear whether the changes of the airway microbiota are cause of consequence of respiratory allergic diseases (Marsland, Nat Rev Immunol 2014). It is needed to discuss this aspect at some point in the manuscript. Discussion 21.-Page 13, line 47-60/page 14 line 1-15: a more detailed and thorough explanation of the immunomodulatory and nervous mechanisms of probiotics is needed. 22.-Page 14, second and third paragraphs are quite redundant, as this information was explained in the introduction. 23.-Page 14, line 46-50: the single-blinded administration of placebos is a regular and ethical procedure for diagnostic purposes in the clinical practice (e.g. food and drug provocations). What it is unethical is the administration of placebo with therapeutic purposes. 24.-Page 16, line 42-46: more studies are needed to establish the adequacy of MRI to assess the allergic inflammation in the nasal mucosa, especially when the technique is not accompanied by a nasal allergen challenge. Moreover, this reference refers to bronchial inflammation, where other techniques (like acoustic rhinometry) are not applicable. Please, rephrase this statement. 25.-Page 16, line 53-55: please, rephrase. This sentence is not clear. The MRI identifies swallowed nasal mucosa, but it cannot establish the aetiology (allergic or other) of the inflammation. Minor points: 1.-Page 2, line 42: elaborate the acronym MRI 2.-Page 6, line 30-31: please, rephrase, the term “additional conditions” is not clear. 3.-Page 7, line 36: elaborate the acronym DAAB the first time it is used 4.-Page 13, lines 8: typo “decepted” 5.-Page 13, line 30-31: please, include a numbered reference. 6.-Page 13, line 32-33/page 16, line 27-29: please, avoid the term “allergies”. Instead, refer to the specific conditions: allergic rhinitis, asthma etc. 7.-Page 14, line 22: “untreated group” rather than “waiting list” 8.-Page 16, line 44-45: “allergic conditions” rather than “allergic reactions”

REVIEWER	Francois Spertini Centre Hospitalier Universitaire Vaudois Lausanne, Switzerland
REVIEW RETURNED	30-Dec-2018

GENERAL COMMENTS	This protocol aims to evaluate the efficacy of a novel probiotic Bifidobacterium longum on allergic rhinitis. One of the originality of this protocol is the presence of three control/placebo arms, one double blinded against verum, one open labeled, and one arm without intervention. There is in my view a number of important weaknesses in this protocol summarized below. 1. Regulatory authorities request a combined symptoms and medication score to evaluate efficacy trials in this domain of allergic rhinitis 2. Use of symptomatic anti-allergy medication needs to be defined 3. There is no indications on how patients compliance will be evaluated 4. Line 12 : carrier should be described 5. MRI will be used as an objective marker of efficacy. It seems difficult to use MRI in a seasonal trial with measurements at start and after one month only. On which basis has the follow up been defined as 4 weeks ? Furthermore this assay is not specific at all and has been demonstrated useful in provocation assays, not in seasonal trials. 6. Birch pollen season start and end should be defined 7. About the composition of the microbiota, it is not clear how much this will relate to the success of the therapy 8. Selection of patients to be enrolled should include a provocation test to demonstrate the severity of allergic rhinitis, possibly a provocation in a pollen chamber. Ideally severity of allergic rhinitis should be evaluated during the season prior to the trial. This seems me of high importance taking into account the aim to evaluate precisely the placebo effect of the treatment. Sera should be tested for IgE at least with a defined minimum level and patients by prick tests. 9. Statistics : percent efficacy of the treatment over placebo in the primary endpoint should be defined, not only the p value for statistical significance

REVIEWER	West, Nicholas Griffith University, Immunology Research Group
REVIEW RETURNED	02-Jan-2019

GENERAL COMMENTS	This is an interesting protocol and addresses the issue that ARS has substantial daily and seasonal variation in symptomatology. The addition of the OLP and nocebo will provide strong information for the design of a phase 3 trial. My key concerns include: 1. Exclusion criteria: Should people who have been on antibiotic use or have a history or GI disorders be excluded? 2. Primary outcome: to be scientifically valid the authors need to choose one primary outcome measure and in the sample size calculation include the size of the difference expected between the probiotic and placebo groups along with the level of uncertainty. The authors note that the primary outcome is the ARS symptom score. Is this the overall score that is the primary outcome or one

	of the individual symptom scores? Please specify in the protocol the specific primary outcome. Further to expressly identifying the primary outcome measure can the authors detail the difference in the change score for the measure on which the sample size is based? Cohen's d is a ratio measure not an expected difference for which a sample size estimate can be based. Is the change in the measure associated with a clinical improvement in symptoms? 3) will the authors be undertaking adjustments for multiple comparison? Minor: Spelling of diatory and microbiom (dietary and microbiome).

VERSION 1 – AUTHOR RESPONSE

Response to reviews

Comments of reviewer #1:

1. „The two objectives of the study are very different an unrelated. It would be desirable to focus only on the effect of probiotics or on the potential of open placebo treatment for allergic rhinitis.“

We understand the point of the referee. However, although two objectives may be confusing at first glance, we think that in this study it makes sense to have two goals. Both objectives profit from each other (e.g., in terms of additional control conditions), and this seems specifically necessary (or at least helpful) with waxing and waning symptoms as in allergic rhinitis. Therefore, we would like to keep both objectives in this protocol.

2. „There is a general confusion throughout the manuscript between allergic rhinitis and chronic rhinosinusitis. These are two different diseases, with different immunopathology and management. The authors probably want to focus on allergic rhinitis, but they refer to “allergic rhinosinusitis (ARS)” throughout the text. This term is not used in the published literature on the topic. Allergic rhinitis patients may have sinusitis episodes as consequence of their nasal inflammation, but the sinusitis in this scenario is a complication rather than a component of the underlying disease. “

We agree with the reviewer and clarified this issue in the revised manuscript. Thus, we now use the term allergic rhinitis throughout the manuscript. (see revised introduction, methods, and discussion).

3. „The authors do not mention in any section the main disease-modifying treatment for allergic rhinitis, which is allergen immunotherapy. This treatment has proven effective for allergic rhinitis, with a level of evidence way stronger than that of probiotics (Calderon, Cochrane 2011) “

We agree with the referee that we should provide this information on allergen immunotherapy. We added in the abstract:

“The main disease-modifying treatment is allergen immunotherapy”

In the introduction (page 4):

“The main disease-modifying treatment is allergen immunotherapy, which has been shown to be effective for allergic rhinitis with a high level of evidence (1)”

4. „It is not mentioned that allergic rhinitis is an IgE-mediated disease “

We agree with the reviewer that this is an important fact. We added on page 4 in the introduction:

“Allergic rhinitis is known to be an IgE-mediated disease (2)”

5. „Page 5, Line 23: last generation anti-HR1 drugs do not induce severe adverse effect like first generation anti-HR1 (Novak, PAI 2016; Church, Br J Dermatol 2010) “

The revised manuscript now addresses this point (page 4):

“Effects for probiotics in preventing allergic diseases have been reported in particular when prescribed during the perinatal period (3). When probiotics are administered later, when the allergic disease is already established, studies often report mixed conclusions (e.g., (4-8)). Thus, it remains unclear if probiotics are effective for allergic rhinitis when this disease is well-known for years.”

6. Page 5, line 27: several studies and metanalysis suggest that probiotics might be effective in preventing allergic diseases if administered during the perinatal period (Elazab, Pediatrics 2013), whereas their therapeutic effect once the disease is established is more limited. Other studies, as indicated by the authors, show therapeutic potential. This controversy should be addressed in the introduction, as a mean to justify the goals of the study..“

We now address this controversy in the introduction (page 5, top).

“Numerous studies demonstrated effects for probiotics in preventing allergic diseases, in particular when prescribed during the perinatal period (3). When probiotics are administered later, when the allergic disease is already established, studies often report mixed conclusions (e.g., (4-8)). Thus, it remains unclear if probiotics are effective for allergic rhinitis when this disease is well-known for years.”

7. „Page 5, 51: please, elaborate what the author mean with “individual sensitivity to environmental allergens”. Do the author have a reference to support this statement?.“

We are now more clear in this statement and changed it to (page 5, top):

“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 (9). Considering this situation - frequent waxing and waning of symptoms - symptomatic therapies of allergic rhinitis are known to be prone to placebo effects (10-15).)”.

8. „Page 5, lines 59-60: this sentence needs a supportive reference.”

We added a reference to this statement (Benedetti, Neuron, 2014).

9. „Page 6, line 56-57: the specific score to measure symptoms of allergic rhinitis should be clarified from the beginning of the methodology section. There are several systems the authors may choose to use (visual analogue scale, Lebel score, total symptom and medication score, among others).

In the revised manuscript we now clearly state the specific score to measure symptoms in the beginning of the methodology section (CSMS, RQLQ) (see now page 6, Methods, first paragraph).

10. „Page 7, line 3: the authors intend to infer the severity of rhinitis symptoms from the changes in the nasal microbiota. Yet a reference is provided, this approach is quite controversial. For the sake of

simplicity, it would be desirable to use the measurement of the nasal microbiota just as an endpoint of the study. Please, explain already how the microbiota will be measured."

We decided not to exam nasal microbiota in this study.

11. „11.- Page 7, line 3-4: using MRI to assess the volume of the nasal cavity will make the study less comparable with other investigations of therapies for allergic rhinitis. Moreover, the authors do not intend to perform a nasal allergen provocation before and after the treatment period. This aspect is a clear limitation of the study, as the current design will not allow differentiate between the nasal inflammation caused by allergens, or by any other trigger (e.g. viral infection). Combining a nasal allergen provocation with one of the standards methods to measure nasal patency (acoustic rhinometry, rhinomanometry) for assessing the response to the intervention would add a lot to the study (Auge, Allergy 2018, Pfaar, Allergy 2018). Moreover, with the current methodology, the treatment period needs to occur simultaneously than the allergen season in order to make sure the patients are exposed to the allergen. This aspect is another limitation, which will create several logistical difficulties. ."

We decided not to use MRI in this study. We see that it may cause logistical difficulties to cover a similar treatment period for all patients. However, given that previous studies successfully managed these problems (e.g., Schaefer et al., 2016, 2018), we think that this point should be feasible.

12. „Page 7, line 23-26: do the authors plan to publish the result in a peer-review journal?."

We now added that the study results will be published in a peer-reviewed journal.

13. „Page 8, line 3-4: Is 4 week a period long enough for the probiotics to induce significant changes? Probiotics are supposed to promote immunoregulatory pathways with the generation of different types of regulatory immune cells (Marsland, Nat Rev Immunol 2014). Please, elaborate and cite articles to justify why this duration is appropriate

We agree with the reviewer that this is an important issue. We now added on page 7 :

"Previous studies reported that similar time periods are effective for this type of probiotic in children with rhinusinusitis (16), and pharmacy recommendations also suggest a minimum of 4 weeks of treatment.".

14. „Page 8, line 21-28: it is not clear if the diagnosis of allergic rhinitis will be confirmed. Do the patients need to have a confirmation of IgE sensitization to aeroallergens (skin prick test or serum allergen-specific IgE)? Do the patients need to be diagnosed by an Allergy sub-specialist, or by any physician? Do the authors intend to include only individuals with seasonal allergic rhinitis, or also with perennial forms of the disease? Please, specify".

We now clarified this issue. The revised version states (page 7 and 8):

"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)."

15. „Page 8, line 31-40: chronic rhinosinusitis, or any other chronic nasal condition (severe anatomical alterations as septum deviation or perforation) should be considered as exclusion criteria."

We followed the suggestion of the reviewer (see page 8, top).

16. „16.-Page 9, line 5-6: please, indicate that these observations are based on mice studies.."

Due to technical reasons we changed the probiotics. Thus, we changed the references to the probiotic.

17. „Page 9, line 27-28 line 58-59: "nasal mucosal samples", the current wording suggests the authors are taking a mucosal biopsy.."

We decided not to exam nasal microbiota in this study.

18. „Page 9, line 52-54: a thorough explanation of which medications will be allowed is needed. Are the authors talking about anti-HR1, nasal corticosteroids, decongestant sprays, or others? How do the authors intend to normalize the intake of symptomatic treatment among the different study groups? This aspect could greatly affect the validity of the results. Alternatively, the study could use the intake of symptomatic medication as an endpoint (e.g. use the combined symptoms and medication score, Rondon, Allergy 2018). Please, replace "sinusitis" by "rhinitis"..."

We followed the suggestion of the reviewers and now include the intake of symptomatic medication as an endpoint using the combined symptoms and medication score (CSMS). Thus, all patients are allowed to continue with their symptomatic medication (anti-HR1, corticosteroids, decongestant sprays etc.). The intake of this symptomatic medication will be used as a measure using the CSMS (see now page 9 and 10).

19. „9.-Page 11, line 1-19: the symptom score the authors intend to use does not seem appropriate, as it grades symptoms related to many other allergic and non-allergic diseases (skin and intestinal conditions) besides allergic rhinitis. This score is not appropriate for the goals and scope of this study..."

See above, point 18. We followed the suggestion of the reviewers and use CSMS as symptom score (see page 9 and 10).

20. Page 11, line 58-60: It is not clear whether the changes of the airway microbiota are cause of consequence of respiratory allergic diseases (Marsland, Nat Rev Immunol 2014). It is needed to discuss this aspect at some point in the manuscript."

We decided not to exam nasal microbiota in this study.

21. Page 13, line 47-60/page 14 line 1-15: a more detailed and thorough explanation of the immunomodulatory and nervous mechanisms of probiotics is needed."

We followed the suggestions of the reviewer and provide now a more thoroughly and detailed description of how probiotics may work (see now revised discussion section, pages 13 and 14).

22. "Page 14, second and third paragraphs are quite redundant, as this information was explained in the introduction.."

In the revised version we changed and reduced these paragraphs in order to avoid repetitions.

23. "Page 14, line 46-50: the single-blinded administration of placebos is a regular and ethical procedure for diagnostic purposes in the clinical practice (e.g. food and drug provocations). What it is unethical is the administration of placebo with therapeutic purposes."

We agree with the reviewer and changed the statements to:

"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 (17)"

"This seems to be very important to profit from beneficial effects of placebos used for a therapeutic purpose in a clear ethical frame." (page 15)

24. "Page 16, line 42-46: more studies are needed to establish the adequacy of MRI to assess the allergic inflammation in the nasal mucosa, especially when the technique is not accompanied by a nasal allergen challenge. Moreover, this reference refers to bronchial inflammation, where other techniques (like acoustic rhinometry) are not applicable. Please, rephrase this statement.."

We decided not to use MRI in this study.

25. "Page 16, line 53-55: please, rephrase. This sentence is not clear. The MRI identifies swallowed nasal mucosa, but it cannot establish the aetiology (allergic or other) of the inflammation.."

We decided not to use MRI in this study.

Minor points:

1.-Page 2, line 42: elaborate the acronym MRI. We removed this sentence.

2.-Page 6, line 30-31: please, rephrase, the term "additional conditions" is not clear.: Done.

3.-Page 7, line 36: elaborate the acronym DAAB the first time it is used. Done.

4.-Page 13, lines 8: typo "decepted".: Corrected.

5.-Page 13, line 30-31: please, include a numbered reference. Done.

6.-Page 13, line 32-33/page 16, line 27-29: please, avoid the term "allergies". Instead, refer to the specific conditions: allergic rhinitis, asthma etc. Done.

7.-Page 14, line 22: "untreated group" rather than "waiting list" Done.

8.-Page 16, line 44-45: "allergic conditions" rather than "allergic reactions" Done.

Comments of reviewer #2:

1. "Regulatory authorities request a combined symptoms and medication score to evaluate efficacy trials in this domain of allergic rhinitis."

See above, reviewer 1. In the revised manuscript we now state that we use a combined symptoms and medication score (CSMS).

2. "Use of symptomatic anti-allergy medication needs to be defined "

The revised manuscript now includes measures to assess medication use during the trial (CSMS). Medication will be categorized according the European Academy of Allergy and Clinical Immunology (EAACI) with respect to H1-antihistaminika, nasal Glucocorticoids, and oral Glucocorticoids (Pfarr et al., 2014, Allergy 2014) (see now page 10).

3. "There is no indications on how patients compliance will be evaluated."

The revised protocol now includes information on how patient's compliance will be assessed. Thus, we added on page 9:

"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."

4. "Line 12 : carrier should be described."

We now provide information about the carrier substance (page 8, bottom).

5. "MRI will be used as an objective marker of efficacy. It seems difficult to use MRI in a seasonal trial with measurements at start and after one month only. On which basis has the follow up been defined as 4 weeks ? Furthermore this assay is not specific at all and has been demonstrated useful in provocation assays, not in seasonal trials.."

We decided not to use MRI in this study.

6. "Birch pollen season start and end should be defined."

We followed the suggestion of the reviewer and now mark the start and end of birch pollen season (see page 7).

7. "About the composition of the microbiota, it is not clear how much this will relate to the success of the therapy"

We decided not to evaluate microbiota in this study.

8. "Selection of patients to be enrolled should include a provocation test to demonstrate the severity of allergic rhinitis, possibly a provocation in a pollen chamber. Ideally severity of allergic rhinitis should be evaluated during the season prior to the trial. This seems me of high importance taking into account the aim to evaluate precisely the placebo effect of the treatment. Sera should be tested for IgE at least with a defined minimum level and patients by prick tests.

We now describe including criteria in this point more clearly. 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 (page 7 and 8) in order to assess the severity of allergic rhinitis.

9. "Statistics : percent efficacy of the treatment over placebo in the primary endpoint should be defined, not only the p value for statistical significance"

In the revised manuscript we now provide more detailed information, including efficacy of the treatment (see also reviewer 3, point 2).

Comments of reviewer #3:

1. "Exclusion criteria: Should people who have been on antibiotic use or have a history or GI disorders be excluded?"

We agree with the reviewer and supplemented the exclusion criteria for patients having used antibiotic medication in the last 6 weeks and gastrointestinal diseases (see page 8).

2. "Primary outcome: to be scientifically valid the authors need to choose one primary outcome measure and in the sample size calculation include the size of the difference expected between the probiotic and placebo groups along with the level of uncertainty. The authors note that the primary outcome is the ARS symptom score. Is this the overall score that is the primary outcome or one of the individual symptom scores? Please specify in the protocol the specific primary outcome. Further to expressly identifying the primary outcome measure can the authors detail the difference in the change score for the measure on which the sample size is based? Cohen's d is a ratio measure not an expected difference for which a sample size estimate can be based. Is the change in the measure associated with a clinical improvement in symptoms?."

The revised protocol now provides more detailed information on this issue. Thus, we clearly indicate the primary outcome measure on which we calculated the sample size. Furthermore, we detailed the difference in change score and the clinical relevance (see page 12).

3. "ill the authors be undertaking adjustments for multiple comparison?

We now claim that we will compute adjustments for multiple comparisons (page 12).

Minor:

Spelling of diatory and microbiom (dietary and microbiome): Done.

Last, since we changed the type of probiotic due to technical reasons, we replaced the probiotic with Ent. faecalis throughout the manuscript. We also changed the title of the protocol.

1. 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.
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.
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4. 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.
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6. 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.
7. 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.
8. 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.
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14. Czubalski K, Zawisza E. The role of psychic factors in patients with allergic rhinitis. Acta oto-laryngologica. 1976;81(5-6):484-8.
15. 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.
16. 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.
17. Blease CR, Bishop FL, Kaptchuk TJ. Informed consent and clinical trials: where is the placebo effect? BMJ (Clinical research ed). 2017;356:j463.

VERSION 2 – REVIEW

REVIEWER	Jalali Mir Mohammad Guilan University of Medical Sciences, Iran
REVIEW RETURNED	21-May-2019

GENERAL COMMENTS	The authors of the paper have in their paper "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" have attempted to design a study protocol to compare the effects of Ent. faecalis with deceptive/open-label placebo/no placebo controls in reducing symptoms of allergic rhinitis. Though the attempt is laudable, I have several issues with the paper. 1- Please do not use the abbreviated word in the title of the article. The title of the article is confusing. Please give it more fluently. Please show that you decide to assess the effects of adding probiotic on standard treatments of allergic rhinitis. It is not necessary to identify all 4-arms of this RCT. Also, title should include the study type (randomized controlled trial). 2- What strain of Ent. faecalis will use in this study? The majority of previous animal study, the researchers measured effects of Ent. faecalis FK-23 on allergic rhinitis. 3- Do you use lysed enterococcus faecalis as oral probiotics? 4- Please insert an appropriate article summary. The majority of previous study were animal study. One of strength point of this study is to assess effects of Ent. faecalis in patients with allergic

	rhinitis. Instead of this point, the article summary principally emphasizes on open label placebo. 5- Page 4, line 39. Literature show that second generation of antihistamines improve the HRQOL. The studies of Meltzer et al. (ref 8, 9) are not about undesirable side effects of antihistamines on the HRQOL. 6- Page 4, line 56. The researchers explained “second generation probiotics”. This term usually to describe probiotics selected based on established mechanisms of prevention or treatment of a specific disease. Is there a second generation probiotic for allergic rhinitis? 7- The aim of publishing protocols is to provide more information about study and to increase transparency for editors, reviewers and readers. Therefore, a large part of the abstract should refer to the method of work and the analysis of the data. 8- The number of trial registration is incorrect. I find only DRKS00015804 at the German Clinical Trials Register which is about a 4-arms RCT using probiotic strain Bifidobacterium longum AH 1206 among patients with allergic rhinitis. 9- Page 5, line 11. Güvenç et al conducted a systematic review and included 22 RCTs. This analysis showed that SMD for total nasal and ocular symptoms with patients with seasonal AR and for nasal QoL scores for studies with LP-33 strain were significant and homogenous. Please consider evidence of systematic review and meta-analysis is not similar to primary studies. 10- It is merit to explain possible effects of Ent. faecalis on allergic rhinitis. 11- Page 5, line 20. El Hennawi et al revealed when stress is controlled by a combined treatment of imipramine and levocetirizine, allergic rhinitis symptoms improved and a better QOL was obtained. It doesn’t mean that symptoms of allergic rhinitis are due to psychological stress. 12- Page 5, line 30. Please rephrase this sentence: “We therefore installed three control groups”. 13- Page 5, line 41. With respect to aims of this study, it is better to omit this paragraph. 14- Page 6, line 26. Please explain the standard treatment of patients. 15- Page 6, line 38. Please describe CSMS and RQLQ shortly. 16- Page 7, line 11. Please Insert “Patient and public involvement” before the result part. 17- Page 7, line 39. The length of this trial is 4 weeks. After the end of trial, all participants will be given probiotics for 4 week. No outcome will be measured after this period and adding of this period shows possible measurement bias of the researchers. 18- Page 7, line 46. Kitz et al prescribed 8 weeks Ent. faecalis probiotics in children with recurrent rhinosinusitis. However, the length of this trial is 4 weeks. 19- Page 8, line 10. Please explain how to assess the severity of the allergic rhinitis. 20- Page 8, line 39. Please omit “biomarkers of allergic rhinitis”. 21- Page 8, line 48. I don’t understand what means this phrase: “To compensate for randomization, placebo provision, and waiting, …”. 22- Please explain about the quantity CFU Ent. faecalis in each ml of Symbioflor-1 suspension. 23- Page 9, line 42. What anti-HR1 stands for?

	24- Page 11, line 8. What EACCI stands for? Please correct "H1-antihistaminika". 25- Page 11, line 27. Please specify criteria for discontinuing or modifying allocated interventions and describe strategies for monitoring adherence to intervention protocol. 26- Page 11, line 30. How many times secondary outcomes are applied? 27- Page 12, line 15. I think that comparison between group 2 and group 3 could not show the size of placebo effect. Also, spontaneous variation could show better in group 4 compared other groups. Although we should be consider that the length of recruitment is about 6 month which could effect on the spontaneous improvement of allergic rhinitis. 28- Page 12, line 25. I have several problems with sample size calculation. First, between 4 cited references (45-48), only study of Costa et al showed significant decreased of the RQLQ global score in the probiotic group compared to control group (difference=-0.29). Second, Lactobacillus probiotics were used in all studies. Third, all subjects in four studies had be diagnosed as perennial allergic rhinitis. Fourth, we want compare the outcomes among four groups in this study. Then, it is necessary to know difference between all groups, i.e., effect size f. Fifth, sample size should be calculated for all primary outcomes. Then, the biggest sample size should be chosen. Therefore, it appears that the sample size is very small with only 30 subjects in each group. 29- Page 12, line 56. Please explain clearer about the random allocation, allocation assignment and allocation concealment. 30- Page 13, line 14. Literature show the beneficial effect of probiotics, mainly lactobacillus paracasaei, on allergic rhinitis. Then, it is not necessary to state it in the discussion section. What is the novelty of this study? To compare probiotics, placebo, open label placebo and no treatment or to assess the effect of Ent. faecalis on allergic rhinitis. Unfortunately, it is not clear. 31- There are several grammatical and syntax errors in the manuscript. This of course can be rectified using appropriate copy-editing. 32- I advise to use the SPIRIT guideline (Standard Protocol Items: Recommendations for Interventional Trials) 2013 for the preparation of this manuscript.

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REVIEW RETURNED	03-Aug-2019

GENERAL COMMENTS	This article has been revised accordingly based on former reviewers' suggestions, and the revised version is more complete and thoroughly. The study design of OLP and no treatment group are reasonable and meaningful, and give readers a clear and acceptable conclusion.

VERSION 2 – AUTHOR RESPONSE

Response to reviews

Comments of reviewer #1:

1. „ Please do not use the abbreviated word in the title of the article. The title of the article is

confusing. Please give it more fluently. Please show that you decide to assess the effects of adding probiotic on standard treatments of allergic rhinitis. It is not necessary to identify all 4-arms of this RCT. Also, title should include the study type (randomized controlled trial).“

We followed the suggestion of the reviewer and changed the title to:

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

2. „ What strain of Ent. faecalis will use in this study? The majority of previous animal study, the researchers measured effects of Ent. faecalis FK-23 on allergic rhinitis.“

We will not use FK-23, but DSM 16440. We now provide this information in the methods section (page 9):

“The probiotic treatment is *Enterococcus faecalis (DSM 16440)”.*

3. „ Do you use lysed enterococcus faecalis as oral probiotics? “

We now provide this information on page 9:

“….cells and autolysate of 1.5 to 4.5 x 10^7 CFU)..”

4. „ Please insert an appropriate article summary. The majority of previous study were animal study. One of strength point of this study is to assess effects of Ent. faecalis in patients with allergic rhinitis. Instead of this point, the article summary principally emphasizes on open label placebo. “

We followed the suggestion of the reviewer and revised the abstract (see new abstract). However, the present protocol has two objectives, therefore we would like to keep the open-label placebo parts.

5. „ Page 4, line 39. Literature show that second generation of antihistamines improve the HRQOL. The studies of Meltzer et al. (ref 8, 9) are not about undesirable side effects of antihistamines on the HRQOL.“

We corrected this point and state:

“Although last generation histamine antagonists do not show severe adverse events anymore (1, 2), current medications for allergic rhinitis may still have some undesirable side effects (3).”

6. “Page 4, line 56. The researchers explained “second generation probiotics”. This term usually to describe probiotics selected based on established mechanisms of prevention or treatment of a specific disease. Is there a second generation probiotic for allergic rhinitis?

We removed this sentence.

7. „ The aim of publishing protocols is to provide more information about study and to increase transparency for editors, reviewers and readers. Therefore, a large part of the abstract should refer to the method of work and the analysis of the data”

We now changed the abstract according to the suggestion of the reviewer and provide more information on method and analysis (see revised abstract).

8. „ The number of trial registration is incorrect. I find only DRKS00015804 at the German Clinical Trials Register which is about a 4-arms RCT using probiotic strain Bifidobacterium longum AH 1206 among patients with allergic rhinitis..”

We corrected the trial registration.

9. „Page 5, line 11. Güvenç et al conducted a systematic review and included 22 RCTs. This analysis showed that SMD for total nasal and ocular symptoms with patients with seasonal AR and for nasal QoL scores for studies with LP-33 strain were significant and homogenous. Please consider evidence of systematic review and meta-analysis is not similar to primary studies.).”

The references here all focus on systematic reviews and meta analyses, but we agree with the referee that we should mention the important study of Güvenc.

In the revised manuscript we now refer to this study more explicitly (see page 6, top):

“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 (4)”

10. „It is merit to explain possible effects of Ent. faecalis on allergic rhinitis..”

We now explain possible effects of Ent. faecalis (page 6):

“Effects of the probiotic Ent. faecalis have been suggested by several studies. For example, it has been demonstrated that Ent. faecalis reduces the number of rhinosinusitis episodes (5). Based on similar studies that report beneficial effects of Ent. faecalis for the immune system (6-11), we hypothesized that this probiotic may also reduce symptoms in seasonal allergic rhinitis.”

11. „ Page 5, line 20. El Hennawi et al revealed when stress is controlled by a combined treatment of imipramine and levocetirizine, allergic rhinitis symptoms improved and a better QOL was obtained. It doesn’t mean that symptoms of allergic rhinitis are due to psychological stress.

We are now more concrete here:

“For example, El Hennawi et al. showed improved symptoms of allergic rhinitis when stress is controlled by a pharmacological treatment (12).”

12. „ Page 5, line 30. Please rephrase this sentence: “We therefore installed three control groups”.

We corrected this statement to:

“We therefore designed a study with three control groups.” (page 6, bottom).

13. „ Page 5, line 41. With respect to aims of this study, it is better to omit this paragraph.“

Given that this study protocol has two objectives (effects of a probiotic and of OLPs on allergic rhinitis), we would like to keep this paragraph (see also our responses to previous referees).

14. „ Page 6, line 26. Please explain the standard treatment of patients.”.

We are now more concrete here (page 6):

“Before and after the treatment (probiotic/placebo, no treatment)…”

15. „ Page 6, line 38. Please describe CSMS and RQLQ shortly..”

We followed the suggestion of the reviewer (see page 7, bottom):

“The CSMS is a simple and standardized method that balances both 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.”

16. „ age 7, line 11. Please Insert “Patient and public involvement” before the result part.

We moved this part to the end of the methods section.

17. „ Page 7, line 39. The length of this trial is 4 weeks. After the end of trial, all participants given probiotics for 4 week. No outcome will be measured after this period and adding of this period shows possible measurement bias of the researchers.”

At the end of 8 weeks we will measure outcome. We are now clearer on this point (page 10):

“…they are asked to provide questionnaire data on symptoms course (outcome measures) over the 4 weeks, but no further site visit in envisioned.”

18. „ Page 7, line 46. Kitz et al prescribed 8 weeks Ent. faecalis probiotics in children with recurrent rhinosinusitis. However, the length of this trial is 4 weeks”

The trial length in the Kitz et al. study was 8 weeks; the length of the present trial is 4 weeks plus a follow-up of additional 4 weeks. Thus, we think that the lengths of the trials are comparable.

*19. „*Page 8, line 10. Please explain how to assess the severity of the allergic rhinitis.“

We will use results of IgE sensitization to aeroallergens as indication for allergic rhinitis, but not to assess severity. We corrected this issue.

20. Please omit “biomarkers of allergic rhinitis”.

Done.

*21. “*Page 8, line 48. I don’t understand what means this phrase: “To compensate for randomization, placebo provision, and waiting, ...”

We corrected this sentence to:

“After the end of the experimental phase we will offer the probiotic for another 4 weeks for all patients (open label probiotic phase).”

*22. “*Please explain about the quantity CFU Ent. faecalis in each ml of Symbioflor-1 suspension.“

We now provide this information (page 9, bottom):

"..(cells and autolysate of 1.5 to 4.5 x 10^7 CFU).."

23. "Page 9, line 42. What anti-HR1 stands for?"

We replaced this term with "antihistamines".

*24. "*Page 11, line 8. What EACCI stands for? Please correct "H1-antihistaminika".

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EAACI stands for "European Academy of Allergy and Clinical Immunology", see page 11 bottom. We corrected the H1-antihistaminika to antihistamines.

Furthermore, we now explain DAAB when first mentioned (page 8).

25. „Page 11, line 27. Please specify criteria for discontinuing or modifying allocated interventions and describe strategies for monitoring adherence to intervention protocol."

We now provide this information (page 12):

"The safety of patients will be monitored at each study visit. Participants will receive a 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."

See also page 10:

"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."

26. „Page 11, line 30. How many times secondary outcomes are applied?"

We provide this information now at the end of this paragraph (page 11):

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

27. „Page 12, line 15. I think that comparison between group 2 and group 3 could not show the size of placebo effect. Also, spontaneous variation could show better in group 4 compared other groups. Although we should be consider that the length of recruitment is about 6 month which could effect on the spontaneous improvement of allergic rhinitis."

We are now more concrete here and corrected the statement to:

"..between group 2 and group 3 for the size of the open-label 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 (open-label) placebo effects."

We agree with the reviewer that the length of recruitment may affect spontaneous improvement. However, we hope that randomization and comparisons with no-treatment group will help us to assess this effect. In addition, we added this point as a limitation in the section “Strengths and limitations of the study” after the abstract.

28. „Page 12, line 25. I have several problems with sample size calculation. First, between 4 cited references (45-48), only study of Costa et al showed significant decreased of the RQLQ global score in the probiotic group compared to control group (difference=-0.29). Second, Lactobacillus probiotics were used in all studies. Third, all subjects in four studies had be diagnosed as perennial allergic rhinitis. Fourth, we want compare the outcomes among four groups in this study. Then, it is necessary to know difference between all groups, i.e., effect size f. Fifth, sample size should be calculated for all primary outcomes. Then, the biggest sample size should be chosen. Therefore, it appears that the sample size is very small with only 30 subjects in each group.“

We now corrected these points. There are no studies examining effects of Ent. faecalis on symptoms (RQLQ or CSMS) of seasonal allergic rhinitis in patients. However, we now refer to more appropriate references, including studies on different probiotics on seasonal allergic rhinitis (points 1-3 of the referee). Furthermore, we made clearer that sample size calculation considers the numbers of different groups, using effect size f (point 4).

With respect to our second aim, the investigation of OLP effects on allergic rhinitis, effects sizes based on previous studies are known. We now supplement this section with this additional calculation.

In addition, we report another study reporting similar group sizes in a probiotic study on immune parameters in seasonal allergic rhinitis.

We added on page 13:

“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 (13-15). (…)

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 (16).

Furthermore, based on previous studies we calculated power calculations on the effect of OLPs on symptoms in allergic rhinitis (17, 18). 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.”

29. „Page 12, line 56. Please explain clearer about the random allocation, allocation assignment and allocation concealment..“

In the revised version we now provide more information here (page 14):

“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."

30. „Page 13, line 14. Literature show the beneficial effect of probiotics, mainly lactobacillus paracasaei, on allergic rhinitis. Then, it is not necessary to state it in the discussion section. What is the novelty of this study? To compare probiotics, placebo, open label placebo and no treatment or to assess the effect of Ent. faecalis on allergic rhinitis. Unfortunately, it is not clear."

As outlined above, this study has two objectives. The first one refers to effects of Ent. faecalis on AR, the second one focuses on the effects of OPL. We are now clearer on this point in the discussion section (page 17):

"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."

31. „There are several grammatical and syntax errors in the manuscript. This of course can be rectified using appropriate copy-editing."

We thoroughly revised the manuscript with regard to grammatical and syntax errors.

32. „I advise to use the SPIRIT guideline (Standard Protocol Items: Recommendations for Interventional Trials) 2013 for the preparation of this manuscript."

We followed the suggestions of the reviewer and the editor and used the SPIRIT guidelines (see SPIRIT sheet attached to this submission).

Comments of editor:

1. "Please revise your title so that it includes your study design. This is the preferred format for the journal."

Done, the new title is „Effects of a probiotic treatment (Enterococcus faecalis) and open-label placebo on symptoms of allergic rhinitis - study protocol for a randomized controlled trial"

2. “Along with your revised manuscript, please provide a completed copy of the SPIRIT checklist (http://www.spirit-statement.org/). Please remember to include the relevant page number(s) from the manuscript next to each reporting item or state 'n/a' next to items that are not applicable to your study.“

Done.

3. “Please improve the strengths and limitations section after the abstract. Please use full sentences and make it clearer why each point is a strength or limitation. Each bullet point should be a single sentence relating to the study’s methods..“

We now included a much more detailed section.

4. Please move the 'Ethics and dissemination' section to after the ‘Methods and analysis’ section (and just before the ‘Discussion’ section) in the main manuscript, as per the journal’s instructions for authors for study protocols.

Done.

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