## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | The effect of a multispecies probiotic on reducing the incidence of antibiotic-associated diarrhoea in children: a protocol for a randomised controlled trial |
| **AUTHORS** | Łukasik, Jan; SZAJEWSKA, Hania |

## VERSION 1 – REVIEW

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| **REVIEWER** | Rune Aabenhus<br>Center for research and education in general practice, Copenhagen University, Denmark |
| **REVIEW RETURNED** | 18-Jan-2018 |

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| **GENERAL COMMENTS** | This is a well written study protocol of an RCT to assess the effectiveness of a novel treatment of AAD with a multiple probiotic strain commercially available in several European countries.<br>The Protocol is prepared according to SPIRIT guidelines and outcomes are appropriate and valid including common and standardized scoring systems for diarrhea. Sample size calculations are done to detect 11% difference between groups. Randomisation, allocation concealment and blinding are well described.<br>My major reservation regards the choice of including a placebo group as comparator when current best practice, if clinically indicated to issue prophylactic treatment for AAD in children, includes use of probiotics. I believe the comparator group should reflect this and include a single strain probiotic such as Lactobacillus rhamnosus GG or Saccharomyces boulardii. Thus a head to head trial would be more appropriate in my view.<br>The authors have addressed this issue on P 7 section Explanation for choice of comparators. They argue that such a head to head trial may overestimate the effect of the multispecies preparation if no placebo group is included. Since the incidence of AAD has been shown to vary greatly between studies (3 to 80%) this is a possibility, but the key question remains if multispecies probiotics are better than current best treatment. Also, this specific concern could be addressed using a three-arm study with a placebo control group. Furthermore, previous research from the University of Warsaw suggests that the incidence is 13,5%.<br>Authors also voice reservations against a head to head trial as this may violate current recommendations on probiotics: e.g. only indicated if there is a clinical reason for their use. However, adverse effects from probiotic treatment have previously been shown to be few and minor and I find it hard to understand this argument in light of providing a “unknown” formulation containing multiple probiotic species to the very same patients. |

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| | Minor comments<br>P 4 line 34 Probiotics MAY confer a benefit.<br>P 5 line 19 please provide the two references for the effect of Ecologic in adults here (first mention)<br>P 9 line 6 : Delete . BY?<br>Funding: Primarily from university of Warsaw. But the manufacturer of the multiple probiotics will provide these free-of-charge, which is already mentioned on page 15. However, in the methods It is described how Winslow will be assisting with the randomization and allocation concealment in the study. This should be mentioned in the funding statement I believe. It is also recommended to describe to whom the data belongs and if the sponsor plays any role in the data analysis, preparation of the manuscript or decision to publish results (which is already mentioned in the ethics and dissemination paragraph). |

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| **REVIEWER** | Stephen Baker<br>OUCRU, Vietnam |
| **REVIEW RETURNED** | 26-Jan-2018 |

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| **GENERAL COMMENTS** | The article “The effect of a multispecies probiotic on reducing the incidence of antibiotic-associated diarrhoea in children: a protocol for a randomised controlled trial” is as it states a study protocol for trial. The protocol is very limited in its content and generally very vague, if the authors wish it to be published they need to add considerably to the procedures and the overall study design. The value of publishing protocols is so that others can perform comparable studies using the same procedures in the future. Specifically, I think the reader need more information on the following criteria before publication.<br><br>General study objectives<br>Definition of treatment failure<br>Consent procedure (which surely should be an inclusion/exclusion criteria)<br>Definition of standard of care<br>Clearly rationale for sample size calculation<br>Benefits and compensation<br>Risk<br>Data and patient confidentiality<br>Data handling<br>Pharmacy and storage of interventions<br>Adverse events (what are they?)<br>Allowed additional medication or definition of protocol violation and withdraw<br>Exact laboratory procedures<br>Sample storage and processing<br>Better analysis plan (may need a statistician)<br>Safety reporting<br>Quality control of therapeutic<br><br>Additional comments<br><br>Do the authors mean probiotic strains or organisms?<br><br>The first 2 strengths and limitations are not specific to the study<br><br>Introduction<br><br>Do you authors mean antibiotics or antimicrobials? |

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| | Incidence is a rate and cannot be referred to as a percentage (prevalence is more accurate but need a denominator)<br><br>Switch between antibiotics and antimicrobials<br><br>Probiotics are not always live<br><br>I think you need to state a very specific aim and hypothesis you are aiming to test<br><br>Methods<br><br>Need to define the study location and not alternatives<br><br>What about consent? Surely proxy consent is required from a parent or guardian, must be an eligibility/exclusion criteria<br><br>Is it 2 doses a day?<br><br>What is the placebo?<br><br>The explanation of comparators needs referencing<br><br>What about ensuring compliance or protocol violations, such as other medications?<br><br>How will you ensure all diarrhea is reported?<br><br>Are these out patients or in patients?<br><br>Study methods, diagnostics laboratory methods and 16SrRNA methods are too vague and no methods are cited<br><br>What are the adverse events?<br><br>The sample size needs more explanation, your sample size is designed to identify a reduction of AAD of 11% in the intervention arm?<br><br>Why are the drug company performing the blinding, and are therefore not blinded, better to get an independent CTU to randomize<br><br>The statistical analysis is weak and would benefit from a study statistician |

**VERSION 1 – AUTHOR RESPONSE**

Editorial Requests:
COMMENT. Can you please add your secondary outcomes to the abstract >> methods and analysis section?
RESPONSE. Done as requested.

COMMENT. Please revise/ improve the 'Strengths and Limitations' section on page 3. You say: “To ensure methodological correctness, the study protocol will follow the rules included in the SPIRIT statement.” SPIRIT is a reporting guideline not a methodology quality assessment. Please also revise the third bullet point. Are there any other strengths relating to the methods/ design of this study?

RESPONSE. Done as requested.

Reviewers' Comments to Author:
Reviewer: 1
Reviewer Name: Rune Aabenhus
Institution and Country: Center for research and education in general practice, Copenhagen University, Denmark
Competing Interests: none declared

COMMENT. This is a well written study protocol of an RCT to assess the effectiveness of a novel treatment of AAD with a multiple probiotic strain commercially available in several European countries. The Protocol is prepared according to SPIRIT guidelines and outcomes are appropriate and valid including common and standardized scoring systems for diarrhea. Sample size calculations are done to detect 11% difference between groups. Randomisation, allocation concealment and blinding are well described.
RESPONSE. We thank the Reviewer for these kind words.

COMMENT. My major reservation regards the choice of including a placebo group as comparator when current best practice, if clinically indicated to issue prophylactic treatment for AAD in children, includes use of probiotics. I believe the comparator group should reflect this and include a single strain probiotic such as Lactobacillus rhamnosus GG or Saccharomyces boulardii. Thus a head to head trial would be more appropriate in my view. The authors have addressed this issue on P 7 section Explanation for choice of comparators. They argue that such a head to head trial may overestimate the effect of the multispecies preparation if no placebo group is included. Since the incidence of AAD has been shown to vary greatly between studies (3 to 80%) this is a possibility, but the key question remains if multispecies probiotics are better than current best treatment. Also, this specific concern could be addressed using a three-arm study with a placebo control group. Furthermore, previous research from the University of Warsaw suggests that the incidence is 13,5%. Authors also voice reservations against a head to head trial as this may violate current recommendations on probiotics: e.g. only indicated if there is a clinical reason for their use. However, adverse effects from probiotic treatment have previously been shown to be few and minor and I find it hard to understand this argument in light of providing a “unknown” formulation containing multiple probiotic species to the very same patients.

RESPONSE. While it is true that a head-to-head trial would directly answer the question “Which probiotic is better?”, we opted for a placebo-controlled trial. First, the use of placebo as a comparator is the gold standard for randomised controlled trials. Second, Lactobacillus GG and Saccharomyces boulardii – two strains with well-documented efficacy – are not available worldwide. For those settings in which none of these probiotics is available, the findings of our trial will be of practical value (if the study product is available).

We agree with the Reviewer that that a three-arm study would have been an optimal model. However, the required sample size would be much higher. This study is performed as part of the PhD project of one of the co-authors (JL). Thus, there are time and financial constraints that preclude such a substantial increase in sample size. Last but not least, the blinding of the three probiotics strains would require cooperation of the manufacturers so that all study products and the placebo would look and smell the same.

COMMENT. Minor comments
P 4 line 34 Probiotics MAY confer a benefit.

RESPONSE. This definition is exactly the same as one recently provided by the International Scientific Association for Probiotics and Prebiotics (Hill C et al. Nat Rev Gastroenterol Hepatol 2014;11:506-14).

COMMENT. P 5 line 19 please provide the two references for the effect of Ecologic in adults here (first mention)
RESPONSE. Done as requested.

COMMENT. P 9 line 6 : Delete . BY?
RESPONSE. Done as requested.

COMMENT. Funding: Primarily from university of Warsaw. But the manufacturer of the multiple probiotics will provide these free-of-charge, which is already mentioned on page 15. However, in the methods It is described how Winslow will be assisting with the randomization and allocation concealment in the study. This should be mentioned in the funding statement I believe. It is also recommended to describe to whom the data belongs and if the sponsor plays any role in the data analysis, preparation of the manuscript or decision to publish results (which is already mentioned in the ethics and dissemination paragraph).
RESPONSE. Done as requested.

Reviewer: 2
Reviewer Name: Stephen Baker
Institution and Country: OUCRU, Vietnam
]Competing Interests: None declared

COMMENT. The article “The effect of a multispecies probiotic on reducing the incidence of antibiotic-associated diarrhoea in children: a protocol for a randomised controlled trial” is as it states a study protocol for trial. The protocol is very limited in its content and generally very vague, if the authors wish it to be published they need to add considerably to the procedures and the overall study design. The value of publishing protocols is so that others can perform comparable studies using the same procedures in the future. Specifically, I think the reader need more information on the following criteria before publication.
RESPONSE. We thank the Reviewer for the thorough review. Please find our reply below.

COMMENT. General study objectives
RESPONSE. Done as requested.

COMMENT. Definition of treatment failure
RESPONSE. Treatment failure is not our outcome measure.

COMMENT. Consent procedure (which surely should be an inclusion/exclusion criteria)
RESPONSE. Done as requested.

COMMENT. Definition of standard of care
RESPONSE. Done as requested.

COMMENT. Clearly rationale for sample size calculation
RESPONSE. Done as requested. Below please find details.

Probability of exposure in controls = 0,16
Probability of exposure in cases = 0,05

Controls per case subject = 1
Alpha = 0,05
Power = 0,8

For uncorrected chi-square test:
N = 121 case subjects and 121 controls

For corrected chi-square and Fisher's exact tests:
N = 139 case subjects and 139 controls

COMMENT. Benefits and compensation, risk
RESPONSE. Done as requested.

COMMENT. Data and patient confidentiality
RESPONSE. Done as requested.

COMMENT. Data handling
RESPONSE. Done as requested.

COMMENT. Pharmacy and storage of interventions
RESPONSE. Done as requested.

COMMENT. Adverse events (what are they?)
RESPONSE. Clarified as requested.

COMMENT.Allowed additional medication or definition of protocol violation and withdraw
RESPONSE. Done as requested.

COMMENT. Exact laboratory procedures. Sample storage and processing
Better analysis plan (may need a statistician).
RESPONSE. The microbiota analysis will be performed as part of an independent study. We have decided to include information on it in the current protocol. However, if the description is considered inadequate, we are prepared to delete this section and report it in a separate study protocol only.

COMMENT. Safety reporting
RESPONSE. We did our best to clarify safety reporting.

COMMENT. Quality control of therapeutic
RESPONSE. As mentioned in the protocol, the study products 'will be produced under Hazard Analysis and Critical Control Point (HACCP) regulations, which is the Dutch regulation system for safety and hygiene in food and food supplements. All components are legally admitted as food additives or food components. Winclove is a NSF International Certified GMP Facility for manufacturing dietary supplements and works with the food safety management system ISO 22000:2005.' Apart from that, no other quality control will be performed.

Additional comments

COMMENT. Do the authors mean probiotic strains or organisms?

RESPONSE. We apologise, but this question is unclear to us. Please forgive our ignorance, but in our opinion both term can be used interchangeably.

COMMENT. The first 2 strengths and limitations are not specific to the study
RESPONSE. Revised.

COMMENT. Introduction
Do you authors mean antibiotics or antimicrobials?
RESPONSE. Revised.

COMMENT. Incidence is a rate and cannot be referred to as a percentage (prevalence is more accurate but need a denominator)
RESPONSE. Revised.

COMMENT. Switch between antibiotics and antimicrobials
RESPONSE. Done.

COMMENT. Probiotics are not always live
RESPONSE. As a matter of fact, the ISAPP definition of probiotics clearly indicates that the probiotic microorganism needs to be live (Hill C et al. Nat Rev Gastroenterol Hepatol 2014;11:506-14). However, we have changed the sentence construction in our manuscript and added quotation marks for clarification.

COMMENT. I think you need to state a very specific aim and hypothesis you are aiming to test
RESPONSE. Done as requested.

COMMENT. Need to define the study location and not alternatives
RESPONSE. Done as requested.

COMMENT. What about consent? Surely proxy consent is required from a parent or guardian, must be an eligibility/exclusion criteria
RESPONSE. Done as requested.

COMMENT. Is it 2 doses a day?
RESPONSE. Correct. As stated in the manuscript: 'The product has a concentration of 2.5*109 CFU/gram, and 2 grams will be given twice daily (total daily dosage of 1x1010 CFU).'

COMMENT. What is the placebo?
RESPONSE. In the revised manuscript, the placebo is described, as requested. We apologise for not doing it earlier.

COMMENT. The explanation of comparators needs referencing
RESPONSE. Done as requested.

COMMENT. What about ensuring compliance or protocol violations, such as other medications?
RESPONSE. Revised, as described above.

COMMENT. How will you ensure all diarrhea is reported?
RESPONSE. For the inpatients, the hospital charts will be checked – this clarification has been added to the manuscript. For outpatients, apart from the study diary, no other method will be employed, as it is not practically feasible.

COMMENT. Are these out patients or in patients?
RESPONSE. Clarified.

COMMENT. Study methods, diagnostics laboratory methods and 16SrRNA methods are too vague and no methods are cited.
RESPONSE. As stated earlier, the microbiota analysis will be performed as part of an independent study. We have decided to include information on it in the current protocol. However, if the description is considered inadequate, we are prepared to delete this section and report it in a separate protocol only.

COMMENT. What are the adverse events?
RESPONSE. Clarified.

COMMENT. The sample size needs more explanation, your sample size is designed to identify a reduction of AAD of 11% in the intervention arm?
RESPONSE. We added an additional explanation to the sample size calculation. The 11 percentage points is a subjective choice of the authors; however, it is based on the results of earlier findings in the trials carried out by our team.

COMMENT. Why are the drug company performing the blinding, and are therefore not blinded, better to get an independent CTU to randomize.
RESPONSE. Indeed, the manufacturer of the study product will perform the blinding. However, the manufacturer will not participate in any other stage of the study. All of the analyses will be performed by our team. As stated in the manuscript, the results of the study will be submitted for publication whether positive or negative. The Reviewer may check PubMed to verify that our group has published both positive and negative trials, and none of our trials (all registered in ClinicalTriala.gov), once finalised, have not been reported.

COMMENT. The statistical analysis is weak and would benefit from a study statistician
RESPONSE. The statistical analysis is similar to the analyses in our previously published studies or protocols (e.g., Aliment Pharmacol Ther. 2005 Mar 1;21(5):583-90; Aliment Pharmacol Ther. 2008 Jul;28(1):154-61; BMJ Open. 2017 Jan 5;7(1):e013928). However, we agree with the Reviewer that some additional analyses may be helpful and have been included that under the revised Statistical Analysis section.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Rune Aabenhus<br>Center for Education and Research in General Practice, Institute of Public Health, University of Copenhagen<br>Denmark |
| **REVIEW RETURNED** | 25-Mar-2018 |

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| **GENERAL COMMENTS** | I have no additional comments |