PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Protocol for a double-blind placebo controlled trial to evaluate the efficacy of probiotics in reducing antibiotics for infection in care home residents - The Probiotics to Reduce Infections iN CarE home reSidentS (PRINCESS) Trial
AUTHORS	Owen-Jones, Eleri; Lowe, Rachel; Lown, Mark; Gillespie, David; Addison, Katy; Bayer, Tony; Calder, Philip; Davies, Jane; Davoudianfar, Mina; Downs, James; Edwards, Alison; Francis, Nick A.; Fuller, Richard; Hobbs, Richard; Hood, Kerenza; Lau, Mandy; Little, Paul; Moore, Michael; Shepherd, Victoria; Stanton, Helen; Toghill, Alun; Wootton, Mandy; Butler, C

VERSION 1 - REVIEW

REVIEWER	Morgan J Katz Johns Hopkins University Baltimore MD USA
REVIEW RETURNED	07-Nov-2018

GENERAL COMMENTS	A well written, well thought out study protocol which is timely and addresses several important questions in a high risk population. The study design is ambitious in an area that is typically very challenging to obtain adequate data. In terms of the primary outcome, I have some concerns that this data may be unreliable. The authors do not specifically describe how antibiotic days will be obtained- review of hospital records from those admitted to the hospital is challenging at best. Can antibiotic administration days be verified with pharmacy records? The authors also do not specify the route of administration of antibiotics- will you specify between IV, oral, and will topical antibiotics be included as antibiotic administration? These are very common in the care home setting. May include some information on training of the RNs who will be abstracting this data- are all RNs part of the research team or will you be relying on RNs from within the care home for weekly diaries or data abstraction? Please comment on whether you will determine and adjust for care home residents who are on probiotics prior to study entry. Consider including as a study limitation: Recent changes in legislature and a push to improve antibiotic stewardship in care home settings may affect the primary outcome through the duration of the 12 month intervention and result in lower CAAD that is unrelated to probiotic administration.

REVIEWER	JC Dionne McMaster University, Hamilton, Ontario, Canada
REVIEW RETURNED	12-Dec-2018

GENERAL COMMENTS	Dear Author, Congratulations on this very interesting protocol. Please see below suggestions for your consideration. 1. Outcomes: Secondary outcomes include diarrhea, and antibiotic associated diarrhea. How will you define diarrhea in this population? How are you tracking diarrhea. How will you diagnosis antibiotic associated diarrhea. Will there be adjudication of the outcomes. 2. Intervention: will residents enrolled be given probiotics/placebo for the study period or only if they are on antibiotics? This could be clarified further in the manuscript. 3. Quality Assurance: will you be doing any testing on capsules to ensure patients are receiving the stated quantity of probiotic species.? Please Clarify 4. Ethics: the consent process could be made clearer. How does the study coordinator establish capacity? Is this standard at your centre? May be made similar with a figure. 5. Mechanistic study: this was exciting to see. Excellent translational research. Why are you checking vitamin D levels? 6. Statistical analysis: modify ITT please justify. 7. Interim Analysis: in the manuscript you state you did perform an interim analysis (page 6, line 45) then state you will not be performing an interim analysis. Please clarify. 8. Style: The manuscript could be streamlined. There are run on sentences that could be shortened.

VERSION 1 – AUTHOR RESPONSE

Comment	Response	Changes made
The study design is ambitious in an area that is typically very challenging to obtain adequate data. In terms of the primary outcome, I have some concerns that this data may be unreliable. The authors do not specifically describe how antibiotic days will be obtained- review of	Many thanks for these comments. We did not have access to pharmacy records. As stated in the manuscript under Primary outcome: “Cumulative systemic antibiotic administration days (CAAD) for all cause infections; total number of days of systemic antibiotic administration as recorded in care home medical records and discharge	The following text has been changed in the manuscript under Diary: Weekly diaries will be collated by the RNs for each participant which will include: level of dose taken of study product and method of ingestion and on what days; and if, during the past week, there have been any signs of infection; use of

hospital records from those admitted to the hospital is challenging at best. Can antibiotic administration days be verified with pharmacy records? The authors also do not specify the route of administration of antibiotics- will you specify between IV, oral, and will topical antibiotics be included as antibiotic administration? These are very common in the care home setting.	summaries if the participant is admitted to hospital, collected by the RNs." And under Diary: "Weekly diaries will be collated by the RNs for each participant which will include: level of dose taken and method of ingestion and on what days; any signs of infection; use of antibiotics; any diarrhoea; hospitalisation; any serious or trial-related adverse event." Registered Nurses (RNs) visit each care home collect data on a weekly basis noting whether, if, during the past week, there have been any signs of infection; use of antibiotics; diarrhoea; hospitalisation and so on. If the answer to any of the prompt questions is 'yes', further information is collected as to which days these covered, and if antibiotics were taken, the route of antibiotic administration is also recorded (e.g. IV, PO, topical, etc.). Information received about antibiotic use while in hospital is captured by the RNs.	antibiotics; any diarrhoea; hospitalisation; any serious or trial-related adverse event. If the answer to any of the prompt questions is 'yes', further information is collected as to which days these covered, and if antibiotics were taken, the route of antibiotic administration is also recorded (e.g. IV, PO, topical, etc.). Information received about antibiotic use while in hospital is captured by the RNs.
May include some information on training of the RNs who will be abstracting this data- are all RNs part of the research team or will you be relying on RNs from within the care home for weekly diaries or data abstraction?	Data collection is undertaken exclusively by Research Nurses (all fully registered with the UK Nursing and Midwifery Council) who are employed by either Cardiff University or the University of Oxford, or by local NHS Research organisations. Care home staff were not involved in data collection.	The following text has been added to the manuscript under Study procedures: Data collection Data collection is undertaken exclusively by Research Nurses (all fully registered with the UK Nursing and Midwifery Council) who are employed by either Cardiff University or the University of Oxford, or by local NHS Research organisations. Care home staff were not involved in data collection.
Please comment on whether you will determine and adjust for care home residents who	An exclusion criterion was "is currently taking regular probiotics and is not willing to adapt to trial protocol" (as stated in submitted protocol paper). Information regarding participants' use	No changes – manuscript lists exclusion of participants currently taking probiotics.

are on probiotics prior to study entry.	of probiotics prior to inclusion in the trial is not being recorded. We excluded participants currently taking probiotics but did not exclude those who may have previously taken them.	
Consider including as a study limitation: Recent changes in legislature and a push to improve antibiotic stewardship in care home settings may affect the primary outcome through the duration of the 12 month intervention and result in lower CAAD that is unrelated to probiotic administration.	Thank you to the reviewer for the suggestion of this limitation, it has now been added. Please bear in mind that this is a placebo controlled, individually randomised trial. Hence external initiatives aimed at enhancing antimicrobial stewardship will affect both arms. The point is well taken, however, overall reductions in antibiotic prescribing might result in fewer end points overall in both arms, resulting in less power than anticipated. We have therefore amended the strengths and limitations points.	Changes made under Strengths and limitations of the study - addition of following bullet point, and deletion of one of the others: Recent antimicrobial stewardship guidance specifically for long-term care facilities could result in lower antibiotic prescribing rates during the trial. Deletion of this text: The intervention and follow-up for up to twelve months in a real-world setting and representative population.
Secondary outcomes include diarrhea, and antibiotic associated diarrhea. How will you define diarrhea in this population? How are you tracking diarrhea. How will you diagnosis antibiotic associated diarrhea. Will there be adjudication of the outcomes.	Many thanks for these comments. Diarrhoea is defined as: 'the abnormal passing of loose or liquid stools, with increased frequency and/or increased volume' (NICE Clinical knowledge summaries). However, the norm for the participant was also considered when collecting this information as care home residents often have loose stools as a result of overflow, aperient use rather than an infective cause. Stool charts are kept in care homes that usually record stool consistency based on the Bristol Stool Chart, and our Research Nurses (RNs) will have access to these charts. We have trained our RNs to record the presence of loose stools. The following items will be analysed (taken from the Statistical Analysis Plan): Antibiotic associated diarrhoea (incidence and cumulative days); all-	The following text has been added to the manuscript at the end of Outcomes: Diarrhoea is defined as: 'the abnormal passing of loose or liquid stools, with increased frequency and/or increased volume' (NICE Clinical knowledge summaries). However, the norm for the participant was also considered when collecting this information as care home residents often have loose stools as a result of overflow, aperient use rather than an infective cause. Stool charts are kept in care homes that usually record stool consistency based on the Bristol Stool Chart, and our RNs will have access to these charts. We have trained our RNs to record the presence of loose stools.

	cause diarrhoea (incidence and cumulative days); mean duration of diarrhoea episodes (i.e. mean duration of consecutive diarrhoea days). We will not be adjudicating any of the outcomes.	
Intervention: will residents enrolled be given probiotics/placebo for the study period or only if they are on antibiotics? This could be clarified further in the manuscript.	As stated under Intervention in original manuscript: "Participants will be asked to take an oral dose of probiotic (LGG and BB-12) or a matched placebo (containing maltodextrin, microcrystalline cellulose, magnesium stearate and silicon dioxide) as a capsule once daily for up to 12-months." This is irrespective of whether they are taking antibiotics or not. We have added a sentence in the manuscript to indicate that the study product will be continued even when on antibiotics and other medications.	The following text has been added to the manuscript under Intervention: Study product will be continued even when on antibiotics and other medications.
Quality Assurance: will you be doing any testing on capsules to ensure patients are receiving the stated quantity of probiotic species? Please Clarify	Nottingham University Hospitals NHS Trust received the bulk probiotic and bulk placebo (in tubes of one month's supply per study participant) in separate boxes from Chr. Hansen. They did their own in-house analyses so they could, if needed, identify them at a later date. Once labelled, samples of study product were cultured to confirm the presence of the probiotic organisms in the active and absence of the study probiotic organisms in the placebo, and this was checked against the allocation codes. These checks confirmed viability for the probiotic as well as correct labelling. The bulk product came with the following documentation: Probiotic: Product information; material safety data sheet; GMO statement; allergen information; BSE statement; Kosher certificate; Halal certificate; nutritional information; product specification; composition statement; safety and origin information for Bifidobacterium (BB-12); safety and origin information for Lactobacillus	No change to manuscript.

	rhamnosus (LGG); certificate of analysis; food GMP statement. Placebo: Product information; certificate of analysis; material safety data sheet.	
Ethics: the consent process could be made clearer. How does the study coordinator establish capacity? Is this standard at your centre? May be made similar with a figure.	The study coordinator does not establish capacity – this is done by the Research Nurse with prior discussion with a senior staff member at the care home. This is covered by the Informed Consent section in the manuscript.	No additional figure added. Addition of text under Informed consent: Where there are concerns that a resident may have impaired mental capacity, a mental capacity assessment will be undertaken by a qualified research nurse (RN) in accordance with the Mental Capacity Act 2005.
Mechanistic study: this was exciting to see. Excellent translational research. Why are you checking vitamin D levels?	This is under the tertiary objectives in protocol: “To determine if the level of serum vitamin D at baseline correlates with colonisation of AMR bacteria in faecal isolates”; this is because vitamin D enhances anti-infective activities of macrophages so it is useful to know vitamin D levels to understand the findings.	The following text has been added to the manuscript under Mechanistic study samples: Vitamin D enhances anti-infective activities of macrophages.
Statistical analysis: modify ITT please justify.	The modified intention to treat analysis maintains a comparison of participants as randomised (regardless of protocol deviations such as non-adherence or contamination). The primary analysis will be based on count data (Poisson or Negative Binomial regression), with an offset variable used to account for the total number of days a participant was observed. Missing data for those remaining in the study is likely to be low, given the high intensity of our data collection strategy, but not all participants will remain in the study for the full 12-months primarily due to death. We therefore believe that this is a sensible primary analysis, as it is randomisation respecting and accounts for the number of observed days. We will also conduct a series of sensitivity analyses that will account for departures from randomised treatment, missing data, and drop-out	The following text has been added to the manuscript at the end of Statistical analysis: A detailed Statistical Analysis Plan (SAP) will be written and signed prior to any analysis commencing, this will detail the modified intention to treat analysis.

	due to death. These will be fully described in our statistical analysis plan, which will be signed off prior to database lock and unblinding. This is covered under: Primary and secondary outcomes analysis	
Interim Analysis: in the manuscript you state you did perform an interim analysis (page 6, line 45) then state you will not be performing an interim analysis. Please clarify.	We performed an "interim assessment" as part of an Internal Pilot Report to determine if we met the Stop/Go contractual criteria stipulated by the funders (successful recruitment; sufficient data collection at three-month assessment; successful analyses of lab samples; interest from further care homes to partake in the trial). We did not perform an interim analysis for the full study.	The following text has been added to the manuscript under Primary outcome:: (to determine if we met the Stop/Go contractual criteria)
Style: The manuscript could be streamlined. There are run on sentences that could be shortened		

VERSION 2 – REVIEW

REVIEWER	Morgan Katz Johns Hopkins University, USA
REVIEW RETURNED	10-Jan-2019

GENERAL COMMENTS	Revision is complete and improves the overall manuscript- this is a timely research protocol that will answer important questions about a vulnerable population.

REVIEWER	JC Dionne McMaster University
REVIEW RETURNED	25-Jan-2019

GENERAL COMMENTS	Dear Authors, Congratulations on your protocol of this very important topic areas. I would recommend being explicit in your definitions of: 1) Diarrhea: how are you going to define this? WHO criteria of diarrhea? Bristol Stool Chart? Given this is a secondary outcome you should clearly define this. 2) C.Diff: again how are you going to define this? Are you going to use a validated definitions such as the IDSA, ACG, SHEA? How will you adjudicate the outcomes. 3)

	Antibiotic associated diarrhea: again manuscript and study could be strengthened by expanding on the definitions. Thank you for submitting this interesting protocol.

VERSION 2 – AUTHOR RESPONSE

Comment	Response	Changes made to manuscript
Diarrhea: how are you going to define this? WHO criteria of diarrhea? Bristol Stool Chart? Given this is a secondary outcome you should clearly define this.	This is defined on P6 of the manuscript at the end of "*Outcomes*": * Diarrhoea is defined as: 'the abnormal passing of loose or liquid stools, with increased frequency and/or increased volume' (NICE Clinical knowledge summaries). However, the norm for the participant was also considered when collecting this information as care home residents often have loose stools as a result of overflow, aperient use rather than an infective cause. Stool charts are kept in care homes that usually record stool consistency based on the Bristol Stool Chart, and our RNs will have access to these charts. We have trained our RNs to record the presence of loose stools.	None
C.Diff: again how are you going to define this? Are you going to use a validated definitions such as the IDSA, ACG, SHEA? How will you adjudicate the outcomes.	As we did not define any patient as suffering from C difficile disease, we did not use the IDSA / SHEA definitions to categorise patients. The study will look at presence of C. difficile in the stool as a risk factor for further disease which could be influenced by probiotics.	The following text had been added to the manuscript: We will look at the presence of *Clostridium difficile* in the stool as a risk factor for further disease which could be influenced by probiotics.
Antibiotic associated diarrhea: again manuscript and study could be strengthened by expanding on the definitions."	Thank you for this suggestion. Antibiotic associated diarrhoea will be defined as diarrhoea occurring following administration of antibiotics and up to eight weeks after stopping antibiotic treatment. (2) (2) Hood K, Nuttall J, Gillespie D, Shepherd V, Wood F, Duncan D, et al. Probiotics for Antibiotic-Associated Diarrhoea (PAAD): a prospective observational study of antibiotic-associated diarrhoea (including Clostridium difficile-associated diarrhoea) in care homes. Health Technology Assessment. 2014;18(63):1.	The following text had been added to the manuscript: Antibiotic associated diarrhoea will be defined as diarrhoea occurring following administration of antibiotics and up to eight weeks after stopping antibiotic treatment (2).