

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Can probiotic yogurt prevent diarrhoea in children on antibiotics? A double-blinded, randomised, placebo-controlled study
AUTHORS	Fox, Michael; Ahuja, Kiran; Robertson, Iain; Ball, Madeleine; Eri, Rajaraman

VERSION 1 – REVIEW

- This manuscript received two reviews at the BMJ but the referees have declined to make their comments public.

VERSION 2 – REVIEW

REVIEWER	Lynne McFarland University of Washington Seattle WA USA Paid lecturer for Biocodex, Lallemand. Scientific advisory board of Bio K+.
REVIEW RETURNED	09-Sep-2014

GENERAL COMMENTS	<p>This RCT assesses the efficacy of a mixed strain probiotic yogurt to prevent AAD in children.</p> <p>Comments:</p> <ol style="list-style-type: none"> 1. Overall, nicely done trial with a solid study design and well-done analysis. 2. Minor comments: Abstract, line 21. Rhamnosus (the species name) should not be capitalized. 3. In Strengths and limitations, page 4/30. 3rd bullet. The names of the last two probiotic strains are still incorrect. Please correct to read: LGG, Lb-12 and La-5. 4. Introduction, line 37-42, the sentence beginning "When sub-group analyses..." this sentence is incomplete or is poorly written. Should be "when ... what happened?" or Rewrite to: Sub-group analyses showed a significant reduction..." 5. Introduction, page 6/30, line 19. Replace the comma after AAD with a period? 6. Methods, page 8/30, line 26. "Youghrt" is misspelled. Please correct. 7. Table 1. Nicely revised. 8. Table 2. This is confusing. Are the row categories NOT mutually exclusive? Are patients counted multiple times? When you add up the numbers with diarrhea in the placebo column, you get a total of 51, but there were only 36 patients receiving placebo. Patients should only be counted once? Please confirm or correct. 9. Discussion. Were your patients inpatients or outpatients? 10. Reference #20. This is an incomplete reference. Please cite the URL or book source.
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	<p>It is unclear how many patients in each group (?/34 in probiotic yogurt vs ?/36 in placebo yogurt) developed AAD. In the discussion, authors state no severe diarrhea in probiotic and 6 in placebo and 1 case of mild diarrhea (diarrhoea-1) in probiotic and 21 in placebo. This is in complete disagreement with what is shown in Table 2. Table 2 shows Diarrhoea-1 (mild?) is 6 in placebo and 0 in probiotic and Diarrhoea-4 (severe?) is 21 in placebo and 1 in probiotic. Which is it? Also, the number in placebo group adds up to 51 not 36 patients enrolled in placebo.</p>
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REVIEWER	Adrian Barnett Queensland University of Technology, Australia
REVIEW RETURNED	27-Oct-2014

GENERAL COMMENTS	<p>This was a clearly written paper describing an interesting and well conducted study.</p> <p>The final bullet points is ancillary to this study "Probiotic yogurt has additional benefit of providing energy and nutrients that are lacking with probiotic supplements/capsules"</p> <p>There are a number of different ways to measure stool frequency and consistency. Were the endpoints used decided on before the results were analysed? And were the cut-points used specified in advance?</p> <p>The paper used a range of diarrhoea definitions in order to increase comparability with the previous literature. Whilst this makes sense it increases the risk of multiple testing of the same hypothesis and therefore a type I error. The results mostly assuage this concern as the differences were large and for all comparisons. However, it might be worth mentioning as a limitation.</p> <p>Why does the Cox survival model need to be corrected for multiple measures? I imagined the analysis would just need one row per patient, with treatment group, time to event and censored (yes/no).</p> <p>Were children censored from the survival model when they dropped out or stopped completing the diary?</p> <p>The study took a very long time to run. A small comment on this would be appropriate.</p> <p>We should never make a statistical comparison between baseline characteristics of randomised groups. A statistically significant test is very likely to be a chance finding (unless randomisation failed). See this paper for a discussion: http://www.sciencedirect.com/science/article/pii/S0140673600020390.</p> <p>You could consider a per protocol analysis based on just those children who complied (in both groups). This might help confirm that it is the differences in the yoghurts causing the effect, and not just the benefits of eating any yoghurt.</p> <p>Minor comments</p> <ul style="list-style-type: none"> - Page 6, I would spell out AAD always as it won't be a familiar acronym to some readers - Page 7, "however the statistical methods were revised before the
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	<p>data analysis was commenced" - can you briefly say why here.</p> <p>- page 7 "During data collection there were only two drop-outs the stool consistency and frequency between the two intervention groups" - something has gone wrong here.</p> <p>- page 8, "Information on prior history was AAD was" first 'was' should be an 'of'</p> <p>- page 10, "compliance for antibiotic and yogurt intake mean" add comma after intake</p> <p>- my first ever analysis (20 years ago) was looking at ways to reduce antibiotic associate diarrhoea, so reading this was very nostalgic!</p>
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VERSION 2 – AUTHOR RESPONSE

Reviewer 1:

1. Overall, nicely done trial with a solid study design and well-done analysis.

Thankyou

2. Abstract, line 21. Rhamnosus (the species name) should not be capitalized.

Un-capitalised. Abstract page 2

3. In Strengths and limitations, page 4/30. 3rd bullet. The names of the last two probiotic strains are still incorrect. Please correct to read: LGG, Lb-12 and La-5.

Corrected to proper spelling as LGG, Bb-12 and La-5. Strengths and Limitations Page 4

4. Introduction, line 37-42, the sentence beginning "When sub-group analyses..." this sentence is incomplete or is poorly written. Should be "when ... what happened?" or Rewrite to: Sub-group analyses showed a significant reduction..."

Rewritten as suggested. Introduction Page 5

5. Introduction, page 6/30, line 19. Replace the comma after AAD with a period?

Period placed. Page 6

6. Methods, page 8/30, line 26. "Youghrt" is misspelled. Please correct.

Corrected to proper spelling. Page 8

8. Table 2. This is confusing. Are the row categories NOT mutually exclusive? Are patients counted multiple times? When you add up the numbers with diarrhea in the placebo column, you get a total of 51, but there were only 36 patients receiving placebo. Patients should only be counted once? Please confirm or correct.

The rows are not mutually exclusive. This has been made clear in the Table. Page 12

9. Discussion. Were your patients inpatients or outpatients?

This information was included in methods section. We have now reiterated this in first sentence of discussion. Page 13

10. Reference #20. This is an incomplete reference. Please cite the URL or book source. Completed and updated. Page 19

11. It is unclear how many patients in each group (?/34 in probiotic yogurt vs ?/36 in placebo yogurt) developed AAD. In the discussion, authors state no severe diarrhea in probiotic and 6 in placebo and 1 case of mild diarrhea (diarrhoea-1) in probiotic and 21 in placebo. This is in complete disagreement with what is shown in Table 2. Table 2 shows Diarrhoea-1 (mild?) is 6 in placebo and 0 in probiotic

and Diarrhoea-4 (severe?) is 21 in placebo and 1 in probiotic. Which is it? Also, the number in placebo group adds up to 51 not 36 patients enrolled in placebo.

We have clarified Table 2 and the definitions of diarrhoea in the text to make clearer. Table 2 page 12 and text page 9

Reviewer 2

This was a clearly written paper describing an interesting and well conducted study.

Thanks very much

The final bullet points is ancillary to this study "Probiotic yogurt has additional benefit of providing energy and nutrients that are lacking with probiotic supplements/capsules"

We have removed this bullet point and included it as a 'may have potential benefit' in the previous bullet point. Page 4

There are a number of different ways to measure stool frequency and consistency. Were the endpoints used decided on before the results were analysed? And were the cut-points used specified in advance?

To remove the ambiguity in measuring stool consistency we used an objective measure to assess stool consistency - the Bristol Stool Scale. The endpoints were decided before any analyses commenced. These endpoints were decided based on the different definitions of diarrhoea in published literature. This information is now made clear in methods. Page 7

The paper used a range of diarrhoea definitions in order to increase comparability with the previous literature. Whilst this makes sense it increases the risk of multiple testing of the same hypothesis and therefore a type I error. The results mostly assuage this concern as the differences were large and for all comparisons. However, it might be worth mentioning as a limitation.

We have calculated a corrected alpha appropriate for multiple independent outcomes. For 8 outcomes the alpha equivalent of 0.05 is 0.033 (approx.). All results reported have p-values less than this corrected value. Thus, the interpretation of the results we believe is correct. In fact the multiple outcomes are not independent, but merely different interpretation of the same observations using different thresholds.

We have presented this in the discussion not as a limitation because it represents an improvement in the data presentation compared to the usual single outcome of presence or absence of diarrhoea.

Why does the Cox survival model need to be corrected for multiple measures? I imagined the analysis would just need one row per patient, with treatment group, time to event and censored (yes/no).

Our mistake. Has been corrected - repeated measures phrase has been removed.

Were children censored from the survival model when they dropped out or stopped completing the diary?

We excluded these patients from the analyses. When we included these two patients in the analyses no change in the estimates of effect was observed. - This statement is included in the results section. Page 12

The study took a very long time to run. A small comment on this would be appropriate. Comment added – this exemplifies the difficulty of recruitment via general/clinical practice in a small town setting. page 14

We should never make a statistical comparison between baseline characteristics of randomised

groups. A statistically significant test is very likely to be a chance finding (unless randomisation failed).

See this paper for a discussion:

<http://www.sciencedirect.com/science/article/pii/S0140673600020390>.

We have removed the sentence from Table 1

You could consider a per protocol analysis based on just those children who complied (in both groups). This might help confirm that it is the differences in the yoghurts causing the effect, and not just the benefits of eating any yoghurt.

Estimates of effect for analyses did not differ between intention to treat and per protocol analyses.

This is indicated in results. Page 12

Minor comments

- Page 6, I would spell out AAD always as it won't be a familiar acronym to some readers.

AAD is defined in Introduction (page 4) and used consistently throughout the text.

Therefore, we have not made the change that the reviewer has requested.

- Page 7, "however the statistical methods were revised before the data analysis was commenced" - can you briefly say why here.

The statistical methods were revised before the data analysis was commenced. To better understand the extent of the gastrointestinal distress, we chose to use different definitions of diarrhoea as the outcome measure and this mandated time to event analyses (Cox proportional hazards regression).

This information has been added to the text page 7

- page 7 "During data collection there were only two drop-outs the stool consistency and frequency between the two intervention groups" - something has gone wrong here.

We have removed this incoherent and redundant sentence

- page 8, "Information on prior history was AAD was" first 'was' should be an 'of'

Changed- was replaced by of Page 9

- page 10, "compliance for antibiotic and yogurt intake mean" add comma after intake Changed-

comma inserted Page 10

- my first ever analysis (20 years ago) was looking at ways to reduce antibiotic associate diarrhoea, so reading this was very nostalgic!

Thank you for sharing

VERSION 3 - REVIEW

REVIEWER	Lynne McFarland University of Washington Seattle, Washington, USA Paid lecturer for Biocodex, Lallemand Scientific Advisory Board for Biok+
REVIEW RETURNED	17-Nov-2014

GENERAL COMMENTS	Nice job of revising! I still would like to know how many children had AAD in the placebo group. Table 2 just gives this data by #episodes of diarrhea by
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	classification and if you add up the number of cases for the placebo, you get 51 episodes in 36 placebo patients. But, still, how many PATIENTS got AAD? Obviously, some children had more than one episode type, but can we at least get this info in the text? Otherwise-great job.
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REVIEWER	Adrian Barnett Queensland University of Technology Australia
REVIEW RETURNED	18-Nov-2014

GENERAL COMMENTS	<p>The authors have answered my queries.</p> <p>In terms of adjusting the p-value, most people would use $0.05/8 = 0.00625$ using the Bonferroni correction, so I'm not sure where 0.033 came from (perhaps another correction method was used). The p-values in table 2 are still under this corrected p-value.</p> <p>Page 30, Line 34, "These included" should be "These were"</p>
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VERSION 3 – AUTHOR RESPONSE

Thank you for giving us the opportunity to submit a revision. We have tried to make the information clear in Table 2 and in discussion regarding reviewer 2 comment