Author's response to reviews

Title: Human lactobacilli as adjuvant given to patients with bacterial vaginosis reduce the recurrence rate after vaginal clindamycin therapy; a 6 month double blind randomized placebo controlled study.

Authors:

Per-Goran Larsson (p-g.larsson@vgregion.se)
Babill Stray-Pedersen (babill.stray-pedersen@klinmed.uio.no)
Kjeld R Ryttig (krr@farmaservice.dk)
Stig Larsen (Stig.Larsen@veths.no)

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Author's response to reviews: see over
Dear Dr Lolu da-Silva  
Assistant Editor, BMC-series journals

Thank you very much for the referee comments. As you said there have been some further criticisms to our manuscript. We have also noted that it is your policy to allow a maximum of two revisions on manuscripts under consideration and thus the next revision is the last on which you are willing to seek advice. You therefore urge us to make every effort to fully address the criticisms during this revision.

Dr Klebanoff wrote that his concerns have been met why we do not comment on that.

Dr Reid have still some concerns. He has new comments are in bold and we have tried to answer his new questions as good as we can but we feel that he in some way do not want to see or understand our earlier answer. And in some we do not understand what he wants. As a matter of fact we have not changed anything in the manuscript (except that we have add a new flow sheet that we had send in before). We have tried to address all his concerns as good as possible.

Secondly, Dr Reid has not written any declaration that he has no competing interests. All other reviewers that BMC have used have written this (I have seen 4 other reviewer this year from BMC). Dr Reid has commercial interest in competitive lactobacilli that are to be sold for treatment for BV. Dr Reid could therefore have interest in that our article is not to be published.

I hope that you will take your time and read our answer and make your own judgment.

If the article is accepted for publication, the cost for this will be paid by:

Annette Sørensen  
Bifodan A/S  
Bogbinderivej 6  
DK-3390 Hundeste  
Denmark  
CVR-No DK11169732

Yours sincerely

P-G Larsson

Reviewer’s report

Title: Human lactobacilli as adjuvant given to patients with bacterial vaginosis reduce the recurrence rate after vaginal clindamycin therapy; a 6 month double blind randomized placebo controlled study.
Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

I have a number of concerns about this paper.

1. The use of lactobacilli does not constitute an adjuvant. This term should not be used.
   THIS WAS NOT ADDRESSED

   As we wrote in our earlier answer; We have used first clindamycin and then lactobacilli why should this not constitute as adjuvant? According to Doraland’s medical definition: adjuvant is a pharmacological agent added to a drug to increase or aid its effect. We could have used the term “Augmentation of clindamycin therapy” instead as Dr Reid have done in his article but for instance in oncology therapy adjuvant are more common used (adjuvant chemotherapy or adjuvant radiation therapy). Then we do not understand the reviewers comment, could he clarify what he means.

2. What was the rationale for repeated vaginal therapy for three months? This does not constitute cure of BV.
   THIS WAS NOT ADDRESSED. SIX MONTHS LATER WHY WOULD THERE BE A DIFFERENCE DUE TO A THERAPY THAT DID NOT WORK AND WAS NOT ABLE TO COLONIZE THE VAGINA?

   Yes this has been addressed. The rationale to treat repeatedly for three month was to see if the recurrence rate could be lower. How would it be possible to investigate this if not by given repeated vaginal therapy? If our lactobacilli could or could not colonize the vagina non of us knows.

3. BV can be caused by aerobic bacteria and Gram positive Atopobium. Please update your knowledge of the condition.

4. Treatment of BV does not reduce the incidence of Preterm labour – not if you look at all the literature.
   THIS WAS NOT ADDRESSED, AND THERE IS A MISLEADING STATEMENT
ON PAGE 5.

We do not agree with the reviewer. As we have stated in our answer there are still controversy about whether or not BV should be treated during early pregnancy.

As we wrote: “The discussion of the connection between preterm deliveries (PTD) (not preterm labour) and BV might be little too long and complex for us to include in this article. Helen McDonald, in her latetest Meta-Analysis (Cochrane from January 2007) draws the following conclusion “However, treatment before 20 weeks’ gestation may reduce the risk of preterm birth less than 37 weeks (Peto OR 0.63, 95% CI 0.48 to 0.84; five trials, 2387 women)” She states that they are updated until September 2006 but our study in the BJOG (from June 2006) is not include in the analysis. It is that study that we use as a reference to our statement. In that article we discuss the difference between extreme preterm deliveries and PTD. PTD before the 37th gestational week is not any big clinical issue but extreme preterm deliveries (before 32 weeks) are”.

There is a big difference between preterm labour and preterm birth. Preterm labour is a woman that will have contractions of the uterus but not having a delivery. Preterm birth is when the baby is born before 37th gestational week. However we stated on page 5 that treatment will decrease the number of extreme preterm deliveries, a statement that we also have a reference to. In that study we treat women before 16th week of gestation so it is in concordance with the conclusion of Helen McDonald’s Meta-Analysis. There have been published more than 19 Meta-Analysis debating the 17 published studies on treatment of BV could lower the PTD. The purpose of our article is not to try to solve, nor to settle this debate.

5. This is not the first study on augmentation of antibiotics with probiotics to treat BV. See Anukam et al. 2006. THEY DO NOT CITE THE PAPER

We cite the following paper; Anukam K, Osazuwa E, Ahonkhai I, Ngwu M, Osemene G, Bruce AW, Reid G: Augmentation of antimicrobial metronidazole therapy of bacterial vaginosisis with oral probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14: randomized, double-blind, placebo controlled trial. Microbes Infect 2006, 8(6):1450-1454.

And we are aware that Dr Reid also published, together with Anukam other papers but we can not cite every paper that Dr Reid has done.

6. The paper by Anukam in late 2006 showed that lactobacilli could cure BV, so the statement on page 5 is incorrect.

9. What is the sample size calculation and why were sexually transmitted infections not ruled out given their high occurrence in BV subjects?

NO SAMPLE SIZE GIVEN

We wrote in our answer:

Our answer we state that “On page 10 we state that “A difference in relapse rate between the lactobacilli group and the placebo group of at least 20% was considered clinically relevant. Power analyses with a significance level of 5% and a power of 80%, 
resulted in at least 46 patients having to be included in each of the two treatment arms to show a 20% difference”.

Well as 46 times 2 will give a sample size of 92 patients we thought that the readers are capable of this simple calculation. Do the reviewer want us to add: “With an 10% calculated drop ut rate we have to include 100 patients into the study”. We can add this line but as Dr Reid say “NO SAMPLE SIZE GIVEN” we are not sure about what he mean.

10. Was BV cured by the time lactobacilli was given? If so, you are using the lactobacilli to prevent recurrence not treat BV.
THIS WAS NOT ADDRESSED.

We say on page 5 that “The primary objective of this study was to investigate if adjuvant lactobacilli treatment could increase the initial cure rate after vaginal clindamycin treatment and secondly if lactobacilli as adjuvance could increase the time to relapse after successful treatment with vaginal clindamycin in patients with BV” i.e. to prevent recurrence”.

After the first treatment of BV with clindamycin we did not control the treatment result before the lactobacilli was given. We are giving lactobacilli both to treat BV and to prevent recurrence.

11. As the lactobacilli used here did not work (see first month’s results – 64% v 74%), you should discuss this. Presumably the strains do not have the appropriate properties to populate the vagina and interfere with the process of BV.
THE FACT THAT THE THERAPY DID NOT WORK IS NOT ADDRESSED

In the discussion we write;
There was, however no improvement in the initial cure rate. The ITT cure rate was 64% (32/50) in the lactobacilli group versus 78% (39/50) in the placebo group. However any patient with missing or unclassified smears at the initial visit who continued the study and whose next smear indicated a cure was included in the cured group and by omitting two of the patients in the lactobacilli group who reported that they did not take any vaginal capsules, the initial cure rate would be 37/48 (77%) in the lactobacilli group, thus there are no difference between the lactobacilli and the placebo group in the initial cure. This cure rate (77-78%) is higher than earlier reported [5] but one reason could be that we report the cure after the first menstruation and not after 28 days. The first menstruation could occur already after 14 days. Another hypothesis is that the antibiotic effect of clindamycin lasts longer than the seven days of treatment. That is feasible if clindamycin concentrates in the vagina though a counter-current circulation similar to that which has been demonstrated for penicillin [18]. In such case, the antibiotic effects of clindamycin would still be present when the first lactobacilli capsules are introduced. The lactobacilli strains are sensitive for
antibiotics such as clindamycin. This could explain that there was no difference in the initial cure rate. A broad spectrum of antibiotics such as clindamycin was chosen as the trial drug since it eradicates all native lactobacilli strains making it possible to introduce new strains of lactobacilli; this is not the case for metronidazole which has no antibiotic effect on the lactobacilli. Presumably it is also possible that the strains used in this study could be upgraded to a strain that more rapidly will increase the restoration of the normal vaginal microbiota.

If this is not discussion about why the therapy does not work then how much must we write to satisfy the reviewer?

12. Were subjects instructed how to take swabs? How was compliance checked?

NOT STATED. DID ANYONE TEACH THE SUBJECTS TO SELF-SWAB AND HOW GOOD AT THIS WERE THEY?

Yes and we have checked this in an other study (Eriksson et al reference number 12 ) why we have added this line.…." according to earlier described method [12]."

If Dr Reid are interested he can read that article. This works very well, in fact from 600 abortion patients were the doctor took the vaginal sample 12 (2%) were not possible to classify were as 400 pregnant women taken self taken samples only 1 (0.2%) was not possible to classify…This is however not published yet.

There is also another reference that we did not use but that we can give to Dr Reid.


13. Bacteria are not roughly recorded. They must be precisely enumerated.

14. Sentence for reference 17 is completely incomprehensible!

15. What is increased discharge?

16. No antibiotics should have been given. If subjects had UTI or other conditions prior to entry, these should have been cured before inclusion of the patients into the BV study.

17. Presumably the 18 candidiasis patients were not included in the BV analysis. Why would resolution of candidiasis have any effect on malodour caused by amine production by BV organisms?

18. Be careful how you report adverse events. How can whiplash ever be perceived to be due to lactobacilli or placebo!! Who got UTI in which group at what time of the study?
THE STRAINS ARE CALLED 'PROBIOTIC', BUT IF THEY SHOW NO BENEFIT TO HUMANS THEY ARE NOT PROBIOTIC.

We do not agree with the reviewer. We do believe that the strains that we used will be of benefit to humans and therefore could be called “probiotic”

ECOVAG IS NOT A DRUG - OR IS IT REGISTERED AS ONE?

EcoVag is not registered as a drug. Application for marketing authorization have been submitted to several European countries.

PLEASE EXPLAIN THE HAY SCORE IN TERMS OF THE AMSEL CRITERIA. READERS CAN’T FOLLOW THIS.

We are not trying to explain the Hay score in terms of Amsel criteria. It is the difference between the Nugent criteria and Hay/Ison that are of interest. From our paper: Larsson PG, Carlsson B, Fahraeus L, Jakobsson T, Forsum U: Diagnosis of bacterial vaginosis: need for validation of microscopic image area used for scoring bacterial morphotypes. *Sex Transm Infect* 2004, 80(1):63-67. (this is our last reference number 21 in the article). We discuss this in full detail. To diagnose BV, Nugent and Hay/Ison have good concordance but regarding treatment studies Nugent have big limitations as we have pointed out earlier.

As we write in the article, We used Hay/Ison scoring for the diagnosis of BV [14, 15] as we earlier encountered limitations in the use of Nugent scoring. This particularly applies to treatment studies and is even more pronounced when clindamycin vaginal cream is the selected treatment. Clindamycin vaginal cream eradicates almost all bacteria in the vagina, and greatly reduces the lactobacilli morphotype bacteria, giving the Nugent score of around 4. This means that the smear can be incorrectly interpreted as demonstrating intermediate flora. On the other hand, if there are only five Gardnerella morphotype bacteria per vision field, the Nugent score would increase from 0 to 3 even when there are more than 500 lactobacilli morphotype bacteria [21]. The Hay/Ison [14, 15] classification is better adapted to follow-up treatment results. This must be taken into consideration when discussing treatment efficacy.

As we have data from both classifications we can make analysis of the difference. This we are doing to do but as we state on page 9, “Each slide was classified by two methods: according to Hay/Ison classification [14, 15] and to Nugent [16]. Dual classifications were made for a comparative study, the findings of which will be published elsewhere.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
There are too many spelling and grammatical errors.
Use ‘microbiota’ instead of ‘flora’. Lactobacillus should be italicized. ‘Motile’ bacteria, not ‘mobile’. Women ‘who’, not women ‘that’. The visit ‘was’ due, not ‘should be’ due. Metronidazole is misspelt on page 5.

**What next?**: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest**: An article of importance in its field

**Quality of written English**: Not suitable for publication unless extensively edited

**Statistical review**: Yes, but I do not feel adequately qualified to assess the statistics.