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.

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Version: 4 Date: 30 June 2007

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. It was possible to work through the manuscript before everyone went on holiday. We have gone through all points from both the referees. We had included an “intent to treat” analysis but as the referee did not find this clearly stated we have clarified this point by moving the first part of the Result to Material and Method where we declare that we will do an ITT analysis. We have also added a flow chart over the study.

Please note that, regrettably, Fig. 1 was uploaded to you twice; as Fig. 1 and also as Fig. 2. The legend to Fig 2 is correct but the graph is not. We have remedied this in this revised version.

Our revised manuscript has also been professionally copyedited to remedy language errors and we believe it to be acceptable for publication.

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

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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.

Version: 3 Date: 8 May 2007

Reviewer: Mark A Klebanoff

Reviewer's report:

General

This manuscript evaluated the use of probiotic lactobacillus vaginal capsules as adjunctive therapy in treating bacterial vaginosis.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. A major concern was that 'intent to treat' analysis was not followed. One of the first principles of analysis is that everyone randomized must be included with their assigned group, regardless of what happens next. According to the section 'follow up', line 5, women who failed to respond to treatment (which, I might add included study capsules) were re-treated and excluded. Since this exclusion occurred after randomization (and indeed, after the first round of treatment), it is totally inappropriate. The authors must re-analyze their data according to accepted principles of trial design.

We report that the initial cure rate was 64% (32/50) compared to 74% (37/50). This is intent to treat analysis. In the discussion section, we report the per protocol results “By omitting two of the patients in the lactobacilli group that reported that they did not take any vaginal capsules, the initial cure rate would be 37/48 (77%) in the lactobacilli group”. We have moved some part to the Material section and some from the Discussion were moved to the Results to clarify this. We have also added a flow-sheet.

The primary objective of this study was to investigate if adjuvant lactobacilli treatment could increase the initial cure rate after vaginal clindamycin treatment after one month and thus the ITT analysis is done on this primary objective. The patients who had BV after one month were re-treated with clindamycin and also lactobacilli but now outside the study protocol as an open study. We will report these results later in another publication.

To clarify this, we moved the fist part of the results section to the M&M where we could stress that we had done an ITT analysis.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

2. The authors emphasize the value of their treatment. They also note the presence of Mobiluncus to be related to success, but do admit the p-value was not significant. However, they give little emphasis to the fact that their treatment had a lower success rate at 1 month, with a p-value comparable to (and in fact, slightly lower than) that of Mobiluncus.

We do not agree. We discuss the initial cure after one month in 232 words (we have added one line according to referee nr 2) we do not think this equates “give little emphasis” while we comment the presences of mobiluncus with 40 words.

3. It appears that the placebo capsules differed from the treatment ones by more than the presence of lactobacilli. The authors should comment on why this is so, and how it might have affected their results.
In the discussion section we have added:

In the active capsules, the excipients lactitol and glucose were chosen both as diluents and as nutritive growth medium for the lactobacilli. Since the placebo capsules should not contain nutritive growth medium, sorbitol was used together with potato starch. Potato starch was selected instead of cornstarch, in the placebo capsules due to our experience that it gives the best capsule-filling ability when combined with sorbitol. The lactobacilli and the placebo capsules had identical appearance. Although we cannot be certain, we do not believe that the active excipients in the placebo capsules would affect the results of the study.

4. Were the definitions of cure agreed upon a priori, or after the study was done?

The definition of cure was stated in the study protocol for LAV-BV-1 study before the study started.

5. More details need to be provided regarding the mechanics of randomization-- who made up the schedule? Did that person have any patient contact? Were the capsules pre-packaged and study personnel simply assigned the next one in sequence? Was randomization done with sealed envelopes? what was the role of the study pharmacy. The authors should refer to the CONSORT statement for more detail.

The randomization was done by Prof Stig Larsen (who is a statistician) in Norway without any patient contact. The capsules were pre-packaged and the study personnel assigned the next one in sequence. This is a placebo-controlled, double-blind trail with identical packaging of placebos and treatment capsules.. All packages must look the same or it would not have been a double-blind study. On page 9 this is stated and we have clarified this by adding the following line. Under Study design “The initial part of the study was open, whereby patients were treated with vaginal clindamycin for seven days; it continued thereafter as a double-blind, randomized, placebo-controlled trial with parallel group design. …” Pharmacia (later Pfizer) only abetted the clindamycin treatment by giving economical support so that we could buy the vaginal clindamycin from the local pharmacy. This part of the study was open so the patients received packages of clindamycin (in Norway; Dalacin vaginal cream).

Did the authors test the blinding of staff (ie after a woman had completed her study visits, ask the staff what they thought that woman was receiving)?

In the discussion section we state: The strength of our study is that all patients were recruited at the same clinic and that 98 of 100 patients were recruited by the same investigator and that all smears and all follow up were done by one investigator. Only one doctor did see all patients at the follow up and only one study nurse were involved in the contact with the patients. These 2 persons could not guess who had received placebo or active drug.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician. Declaration of competing interests:
I declare that I have no competing interests
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.

Version: 3 Date: 11 May 2007

Reviewer: Gregor Reid

Reviewer's report:

General
There are so many spelling and grammatical errors, it would take me a lifetime to write them all out! In future, please have someone review before submission.

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.

   We have used first clindamycin and then lactobacilli why should this not constitute as adjuvant? According to Dorland's medical definition: adjuvant is a pharmacological agent added to a drug to increase or aid its effect.

2. What was the rationale for repeated vaginal therapy for three months? This does not constitute cure of BV.

   We investigated the cure after one month. Women who had a Hay/Ison score of 3 were given new treatment with clindamycin. Women who were cured were given lactobacilli for 3 month in order to see if treatment with lactobacilli could lower the recurrence rate.

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

   We are very well updated on this condition and we know that BV can be caused by both aerobic and anaerobic bacteria as well as the newly described Atopobium vaginae (Ferris, BMC Infectious diseases 2004). Atopobium vaginae have been associated to BV and A. vaginae are described as metronidazole resistant. But in this introduction we only state that the BV is a disease with unknown aetiology, characterized by loss or reduction of lactobacilli and increased number of anaerobes and Gram-negative rods. We have changes this to "BV is a disease with unknown aetiology, characterized by loss or reduction of lactobacilli and increased overgrowth of other bacteria". Then we do not state whether or not the bacteria are gram positive or negative or Gram variable.

4. Treatment of BV does not reduce the incidence of Preterm labour – not if you look at all the literature.

   The discussion of the connection between preterm deliveries (PTD) (not preterm labour) and BV might be a little too long and complex for us to include it in this article. Helen McDonald, in her latest 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 included in the analysis. It is that study which 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.

5. This is not the first study on augmentation of antibiotics with probiotics to treat BV. See Anukam et al. 2006.
Yes it is the second. We have changed this.

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

We have deleted this statement on page 5.

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

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”.
We did rule out sexually transmitted infections as we found no trichomonas or gonorrhea and only three *C. trachomatis* infections.

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

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.

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.

We have added the following line “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”.

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

Yes, and we have checked this in another study (Eriksso n et al. reference number 12) so we have added this line….” according to earlier described method [12].”

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

Doing microscopy it is not possible to precisely enumerate bacteria. Doing Nugnet scoring the bacteria are divided in 0, 0-1, 1-4, 5-29 and >30 per vision field. When doing microscopy on BV smears there are most of the time between 5 000 and 10 000 bacteria per vision field and in some cases up to 100 000 bacteria per vision field. With this high number of bacteria it is not possible to be precisely enumerated. We have changed the line to: A rough count of bacteria was recorded...

14. Sentence for reference 17 is completely incomprehensible!

This sentence has been deleted. We have added a new explanation in discussion

15. What is increased discharge?

The initial symptoms” were 86% malodorous discharge, 70% increased discharge, 14% itching, and 11% burning as the women themselves reported this. We had no other definition of this than the patients' own.
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.

Of course, No subject had UTI prior to the entry and in a 6 month trial we could only report treatment that her GP prescribed her during our follow up. We report that antibiotics did not affect the result of the relapse of BV during the follow up period.

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?

This is a very interesting question. To recruit patients with symptomatic BV we used advertisements in the local newspaper. Totally 146 patients came to our clinic with malodours discharge. Of these 18 had large amounts of candida albicans observable in her smear with dense lactobacilli and low ph of 4.0. Malodour is subjective and not always caused by amine production of 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?

In all pharmaceutical studies (GCP) all Adverse events (AE) have to be reported. Even whiplash. Then we report the AE that were considered as probable. We have deleted “most frequently” in the line “The reported events were headache, menorrhagia, haemorrhoids, infl…” as this could be misunderstood.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Use ‘microbiota’ instead of ‘flora’.

OK done

Lactobacillus should be italicized.

Yes but that line has been deleted according to #6.

‘Motile’ bacteria, not ‘mobile’.

OK done

Women ‘who’, not women ‘that’.

OK done

The visit ‘was’ due, not ‘should be’ due. …

OK done

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Discretionary Revisions (which the author can choose to ignore)
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.