Reviewers report

Title: Identification and characterization of vaginal lactobacilli from South African women

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Reviewer: Sujatha Srinivasan

Reviewers report:

The manuscript by Pendharkar et al. describes the lactobacilli present in a group of 30 South African women using cultivation based techniques to isolate the organisms and 16S rRNA gene typing to identify the Lactobacillus isolates. They report that profiles are similar to those detected in European populations with Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus jensenii and Lactobacillus iners being the most commonly isolated lactobacilli. The study was conducted in an effort to determine whether Lactobacillus profiles in South African women are similar to European and North American women as a prelude to a probiotic approach to manage the common condition, bacterial vaginosis.

Major Compulsory Revisions

Through the entire manuscript women with BV are referred to as unhealthy and women who don’t have BV are referred to as healthy. There is much debate in the field about the dysbiotic condition BV, although it is true that many studies have shown that BV is associated with increased risks with many adverse health outcomes such as HIV acquisition or preterm birth. Hence, it would be preferable to re-write such that associations are made with the presence or absence of BV rather than healthy/unhealthy. For example:

Lines 45-46: Can be re-worded to: “L. crispatus was significantly associated with the absence of BV (p=0.024).

Line 132: It is unclear from the manuscript if the swabs reached the wet lab within 48 hours post collection and were plated right away or if the swabs were plated within 48 hours after arrival at the lab. Please clarify. If the samples were not plated within 48 hours after collection, then this can have implications on isolation of strains. Were any pilot studies conducted to compare strains isolated after storage for different times? Regardless, this point needs to be addressed under limitations.

Likewise, in 21 women only one type of Lactobacillus species was isolated. This is unusual as typically more than one Lactobacillus type can be cultivated from a vaginal sample when plated within 24 hours. The advantage of cultivation-based approaches is that they facilitate detection of low numbers of bacteria. This is in contrast with molecular approaches such as broad-range PCR with pyrosequencing which tend to bias towards the more abundant sequence types and provides an estimate of relative abundance.
Figure 1 and Table 1 essentially have the same data except the numbers are cumulated in Figure 1. This does not add any significant additional value.

Minor Essential Revisions

Line 32: This is the first time the bacterium is mentioned. Change to Lactobacillus crispatus from L. crispatus and so on.

Line 32: There was no Lactobacillus rhamnosus isolated as you state in the abstract. I presume you are referring to L. ruminis.

Line 35: Please re-phrase. Sentence does not read correctly. Also please state how the Lactobacillus species were identified such that the reader can understand the value of the data presented (Eg. Identified by cultivation and 16S rRNA gene typing).

Line 54: “It is thus likely that…restore normal microflora.” Please remove from the abstract. This is a discussion point, not a conclusion.

Line 78: Please amend to say that “The vaginal microbiota in women is often dominated by lactobacilli.” There may be exceptions.

Line 85: Reference 6 is incorrect for the prevalence of BV. The most recent reference that I am aware of looking at prevalence in the US is Koumans et al. 2007 Sex Trans. Dis. 34:864-869. Likewise, please cite primary references for prevalence in Europe and Africa.

Line 149: Although, it is stated that previously described techniques were used in the amplification and sequencing of the 16S rRNA gene, it would be useful to have a sentence briefly describing the method. Which region of 16S was targeted and how much sequence was obtained?

Line 173: Sentence confusing – please re-phrase.

Line 184: In the methods, the study enrolled 40 women. Were no lactobacilli isolated from 10 women? If so, was it because they had BV? Please provide an explanation. It would be very helpful to have a Table 1 describing the study participant characteristics and demographics of the study population. Please include data such as: prevalence of BV in the cohort, menses at time of collection, use of vaginal products or antibiotic use prior to collection of samples etc. This would alleviate confusion such as the question above on why lactobacilli were isolated from just 30 women in spite of enrolling 40 women in the study.

Lines 185-187: On first reading, I understood the sentence to be: L. crispatus, L. iners and L. gasseri were the dominant Lactobacillus species in 10, 8 and 7 women respectively rather than L. crispatus, L. iners and L. gasseri were present in 10, 8 and 7 women and represent the most common Lactobacillus species isolated from South African women. Please edit for clarity.

Line 192: Edit to Lactobacillus colonization and BV status rather than health
status

Lines 197-201: L. crispatus was isolated…This sentence is confusing to read. Please edit to something like: L. crispatus was only isolated from women without BV. Other predominant lactobacilli (…) were isolated from women with and without BV, but were more often isolated from women who did not have BV.

Lines 220 – 224: Please re-phrase. Confusing.

Line 235: It is either the vagina or the genital tract, not the vaginal tract.

Line 402: Re-phrase to: Association between colonizing lactobacilli and BV status defined by Nugent Score

Discretionary Revisions

While microflora is the terminology that has been often used in the literature, the current trend to refer to the bacteria in/on humans is “microbiota.”

Lines 83-84: While there is a decrease in the numbers of lactobacilli, it would be more accurate to state something like “there is a shift in the microbiota from mostly lactobacilli to diverse anaerobes, which is associated with the common condition, BV.”

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

Declaration of competing interests:

I declare that I have no competing interests.