Title: The influence of probiotics on genital high-risk human papilloma virus clearance, associated lesions and quality of cervical smear: A randomized placebo-controlled trial

Version: 0 Date: 25 Mar 2019

Reviewer: Jo-Ann S. Passmore

Reviewer's report:

The study by Ou et al. describes a randomized, double-blinded, placebo controlled trial to evaluate the efficacy of a Lactobacillus rhamnosus GR-1 and L. reuteri RC-14-containing oral probiotic in clearance of high-risk HPV infections (evaluated by Hybrid Capture II) in 121 women with HR_HPV infections at enrollment, followed up for 15 months. While the oral probiotic treatment did not appear to influence rate of HPV clearance in the study group, the probiotics did influence cervical disease clearance (LSIL) in the 21 women who had cervical disease at enrollment. Overall, the study is interesting although some important details and limitations should be added.

1. Why was vaginal pH or BV status not reported in the study? Since these are acknowledged by the authors to be important variables in HPV risk, it seems appropriate that this information would be collected? If it wasn't, this should be acknowledged as a limitation of the study design.

2. Please justify selection of 50% power for power calculations. This is considerably lower than a power of 80% that is the minimum that should be used for clinical trials. Thus, they were underpowered to show a difference between the groups, and I think their negative result may be misleading. Furthermore, the main positive result from this study is the improved clearance of LSIL. The study certainly was not powered for this outcome, with only 21 women having LSIL.

3. Probiotic was given orally. Justify why U-relax was selected as the product to be used? Please justify why oral administration was selected if vaginal colonization was intended? Please provide more detail about the U-relax product: dose, co-formulation, QC that was done on the product to ensure organisms were live, how long the women were intended to take the probiotics for and what proportion of them reported regular dosing/incomplete dosing? Was antibiotic treatment (for any condition) noted? As this would influence probiotic viability?

4. They don't mention how the placebo was manufactured. Please provide details of this.
5. They only mention ethics approval but no regulatory approval for the trial. Can this be clarified? Who did the randomization? Please provide more detail on inclusion/exclusion/screening criteria.

6. About a third of the cohort were post-menopausal. Authors should comment on whether these women were on hormone replacement therapy? Comment on BV and HPV prevalence post menopause and whether low estrogen levels would influence probiotic efficacy?

7. Discussion - page 10 line 7-10. Please provide evidence that oral probiotics make their way through the GI tract after oral administration to colonize the genital tract in women?

8. Comparison is made in the discussion with the Verhoeven study - please provide more detail about probiotic product used by Verhoeven, dose, duration, strains etc.

9. Discussion page 11 line 14 - authors state that probiotics may influence different strains of HPV differently. Please provide more justification for why you think this would be likely?

10. Limitations section in the Discussion needs to be expanded to include/acknowledge other limitations: probiotics were orally delivered, vaginal colonization was not assessed, BV status was not assessed by standard methods, vaginal pH? Study not powered for the LSIL finding since numbers were so small? Commenting on whether individuals took their probiotics for the full duration of the study or whether this information was even collected? Comment on the fact that the isolates in the probiotic were not vaginal isolates? Ie, were they appropriate for a vaginal probiotic study? HPV was measured by Hybrid capture that is not type specific, and semi-quantitative.

11. Please include information about ASCUS/LSIL prevalence in Table 1.

Minor comments:

Page 8 line 58 - what confounding factors were adjusted for in the multivariate logistic regression analysis?

Page 10 line 20 - Many studies have reported…. They only cite one study. Please correct this.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No
Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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