Reviewer's report

**Title:** Association between cigarette smoking and the vaginal microbiota: A Pilot study

**Version:** 1  
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**Reviewer:** Lenka Vodstrcil

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Association between cigarette smoking and the vaginal microbiota: A Pilot study  
Rebecca M. Brotman et al.

Thank you for the opportunity to review this paper which investigated the association between smoking and vaginal microbiota and found that smokers had an increased odds of having a vaginal microbiota dominated by BV-associated bacteria and lacking protective Lactobacillus spp.

**Strengths:**

The paper has a number of strengths in that it provides pilot data for looking at the effects of smoking on the vaginal microbiota and demonstrates in a very small number that smoking cessation may lead to a reverse of this. Although this is a pilot study of a relatively small group of women, the authors describe in great detail the methodologies and outcomes for each individual participant.

**Revisions:**

The authors may wish to consider some of the following points:

Comment 1: Methods pg 7, ln 138

The authors describe that the region which they applied pyrosequencing to was the V1-V3 hypervariable region that has been previously described. As this results in a long PCR product, is it possible that the PCR was not as efficient as it may be if the region was shorter? Furthermore, are there any known primer biases using this particular set – i.e. could some bacteria be over/under represented?

Minor revisions:

1: Introduction pg 3, ln 42

Before introducing the intervention of smoking cessation, further explanation of other interventions and their inability to decrease the risk of BV recurrence may strengthen the argument that smoking cessation is an important intervention option.

2: Methods Pg 5, ln 94-97

Please consider revising the sentence describing the additional criteria, which
was slightly confusing.

3: Results Pg 9, ln 180
The authors could consider expanding the description of the distribution of all CST groups and their differences in smokers vs non-smokers (i.e. fewer smokers had CSTI dominated flora).

4: Results Pg 9, ln 183
Although smokers were significantly more likely to have increased Nugent Scores compared with non-smokers, their vaginal pH was not statistically different to non-smokers so the authors should consider reporting specific percentage values where differences exist and state that these were not significant.

5: Results Pg 9, ln 187
The authors could include results on the differences in number of sexual partners between smokers and non-smokers considering they then adjust for this variable in the multivariate analysis. Number of lifetime partners has often been associated with increased risk of BV so this is potentially an important observation.

6: Discussion, Pg 11, ln 228-231
To help distinguish between the initial pilot and the smoking cessation pilot, consider re-wording the second sentence in the Discussion.

7: Discussion Pg 12, ln 237-239
It is a little unclear in this sentence what the authors mean about heterogeneity and if it is in any way related to the increased risk to STIs and/or BV.

8: Discussion Pg 12, ln 248
Consider using the word ‘future’ study instead of ‘a’ study.

9: Discussion, Pg 13, ln 258
The authors comment that racial and ethnic differences may affect biomarkers of smoking exposure. How do their pilot data relate to this comment?

10: Discussion, Pg 13, ln 270
The authors correctly state that smokers may have reduced usage of hormonal contraception due to WHO guidelines in which it is recommended that women who smoke and are >35 are not prescribed the OCP. In this pilot study however, the smokers were also more likely to be >40 and therefore are less likely to use the pill anyway if they are peri-menopausal. The relevance of the overlap of these findings could be discussed further.

Similarly, reduced HC-use in smokers may also be attributed to a higher rate of Nugent scores indicative of BV described in line 274. Further discussion could be included.
11: Table 1
Please consider including the statistical test used in the footnote as well as bolding significant values.

12: Table 2
Please consider including the number of women who fell into each variable category and rearrange so that the 95% confidence intervals are reported before the p-values. The authors could also include the statistical test used and bold the significant p-values in the footnote.

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

**Quality of written English:** Acceptable

**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