Quantitative analysis of initial adhesion of bacterial vaginosis anaerobes in ME-180 cells

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Bacterial vaginosis (BV) is the most common vaginal disorder in women of reproductive age [1]. Despite decades of research, the etiology of BV still remains elusive. It is well established that adhesion to host cells or tissues is a necessary early step in the establishment of infection [2]. Since it is often considered a polymicrobial condition, it has been proposed that some bacteria have a preponderant role as early colonizers, while others have an impact later in the development of a multi-species biofilm infection [1]. So, we tested this hypothesis by first quantifying the potential of known anaerobic bacterial species found in BV (*Gardnerella vaginalis*, *Atopobium vaginae*, *Mobiluncus mulieris*, *Prevotella bivia* and *Fusobacteria nucleatum*) to compete with a protective lactobacillus (*L. crispatus*) for initial adhesion to ME-180 cervical epithelial cells. Then, we tested the potential of the two vaginal lactobacilli; *L. crispatus*, which appears to be protective against BV, and *L. iners*, which does not protect as effectively against BV, to block these BV-associated anaerobes. And finally, we tested the ability of the BV-associated anaerobes to displace pre-adhered lactobacilli. This work allowed quantification, of the initial adhesion of these BV-associated anaerobes to ME-180 epithelial cells and competition and displament/blockage assays.

In competition assays, *G. vaginalis* exhibited the greatest capacity for adherence to ME-180 cells, confirming our previous report [3]. Interesting, *G. vaginalis* also maintained its ability to adhere in the presence of *L. crispatus* better than the other species, and there was only a 10% reduction in adherence with respect to the control. This was statically different from the others BV anaerobes (ANOVA Tukey statistical test values, $P < 0.05$), as it adhered approximately 4-fold better than *A. vaginae* or *M. mulieris* and approximately 2-fold better than *P. bivia*. Adherence of *L. crispatus* was not statistically inhibited by any of the BV anaerobes tested.

In order to simulate the introduction of BV-associated bacteria into a healthy vagina colonized by lactobacilli, we performed displacement/blockage assays in the presence of ME-180 cell line. To this end, we first allowed *L. crispatus* or *L. iners* to adhere to the epithelial monolayers and subsequently added a BV-associated species to quantify the inhibitory effect of the lactobacilli on secondary colonization. In these displacement/blockage assays, *L. crispatus* inhibited adherence of *G. vaginalis* by approximately 43%. Addition of *G. vaginalis* appeared to cause a slight displacement of adherent *L. crispatus* but this did not reach statistical significance. *L. crispatus* also reduced adherence of *A. vaginae* and *M. mulieris* by approximately 50%. *P. bivia* and *F. nucleatum* appeared to be less susceptible to inhibition by *L. crispatus*. Interestingly, *L. iners*, which has been shown in previous studies to be less protective against BV relative to other vaginal lactobacilli [4], had a similar inhibitory effect on adherence by all of the BV-associated species except *G. vaginalis*. Adherence of *G. vaginalis* actually increased somewhat in the presence of *L. iners*, although this increase did not reach statistical significance. None of the BV anaerobes displaced *L. iners*.

To conclude, *L. crispatus* and *L. iners* inhibited adherence of all BV-associated species tested with the exception that *L. iners* actually enhanced adherence of *G. vaginalis*. This study supports the possibility that *G. vaginalis* could be an initial colonizer in BV etiology.

Keywords: *Lactobacillus* spp.; *Gardnerella vaginalis*;Bacterial vaginosis; ME-180 epithelial cells

References

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