

P242. *Gardnerella* spp. pre-conditioned vs competitive multi-species biofilm growth and the impact on the tridimensional biofilm structure

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Background

Bacterial vaginosis (BV) is one of the most common bacterial vaginal disorders among women of reproductive age. The hallmark of BV is the presence of a multi-species biofilm, formed primarily by *Gardnerella* spp., in a minor part by *Atopobium vaginae*, and also other anaerobic species. While a few studies have demonstrated that some BV-related species establish synergistic interactions with *Gardnerella* spp. *in vitro* dual-species biofilms, little is known regarding bacterial interactions in triple-species BV-associated biofilms. We evaluated the interactions and spatial distribution of *Gardnerella* spp., *A. vaginae* and a third BV-associated species, such as *Enterococcus faecalis*, *Lactobacillus iners*, *Mobiluncus curtisii*, *Peptostreptococcus anaerobius*, *Prevotella bivia*, and *Staphylococcus hominis*, using two distinct *in vitro* biofilm formation models.

Method

We analyzed the synergistic or antagonistic interactions in triple-species biofilms formed by two distinct experimental designs: one model mimicked the hypothesis that *Gardnerella* spp. is the early colonizer and allowed a mono-species *Gardnerella* spp. biofilm to be formed for 24 h before inoculating the other bacterial species; alternatively, in the second model, all three bacterial species were inoculated simultaneously, i.e. in a competitive way, and incubated for 24 h. Fluorescence *in situ* hybridization with specific peptide nucleic acid probes for *Gardnerella* spp. and *A. vaginae*, and DAPI staining were used to characterize the established biofilms. In addition, quantification of the biofilm mass by crystal violet was performed.

Results and Conclusion

Confocal microscopy data revealed distinct morphologies, but also some similarities, between the triple-species biofilms formed in both models, with a bigger diversity of ecotypes in the top layers of the biofilms, while the bottom layers were more conserved. However, biofilm quantification showed almost similar total biofilm biomass for both models, except for the consortium of *Gardnerella* spp., *A. vaginae* and *S. hominis* which presented higher biomass in the competition model.

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