Development and treatment of a novel *in vitro* biofilm model of bacterial vaginosis

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Background

Bacterial vaginosis (BV) affects 30% of women of childbearing age in the western world, presenting with 3-5 times increased risk of miscarriage and two-fold risk of pre-term birth[1]. Antibiotics such as metronidazole and clindamycin are current therapies, however success rates are low due to the recalcitrance of biofilms consisting of BV-associated bacteria (BVAB) including *Gardnerella vaginalis*. One novel therapy is the use of bacteriophage-derived endolysins which target *G. vaginalis* (Fig1)[2,3], although the efficacy against multi-species biofilms remains unknown.

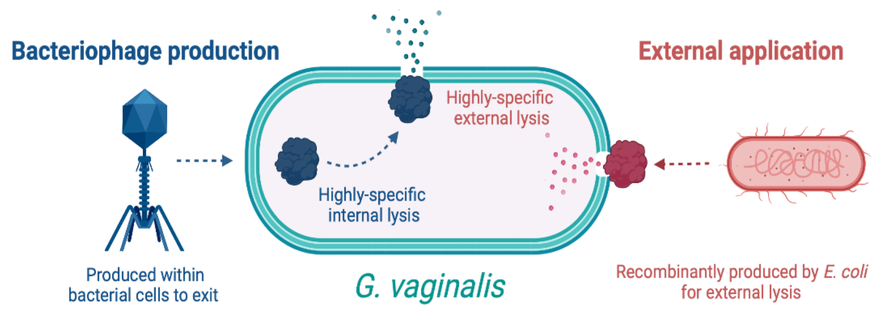


Figure 1: Endolysin internal and external bacterial lysis

Study aim: Screen a novel endolysin therapy against a polymicrobial *in vitro* biofilm model representative of BV.

Methods

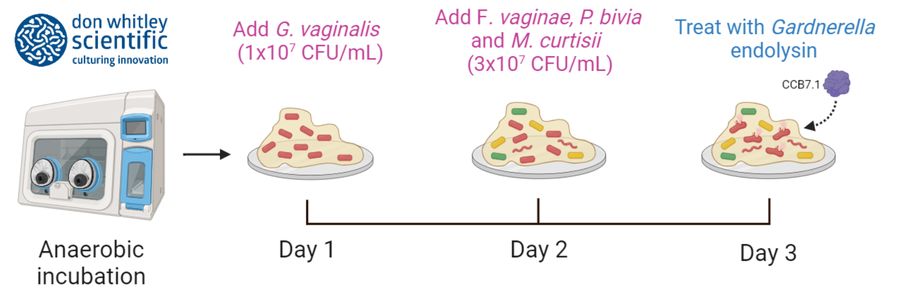


Figure 2: Development of BV-associated biofilm model

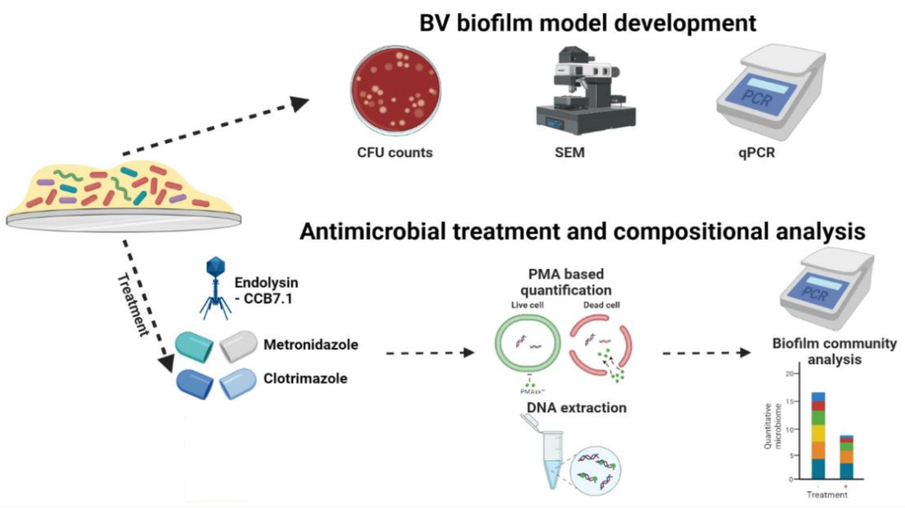


Figure 3: Experimental outputs

Discussion

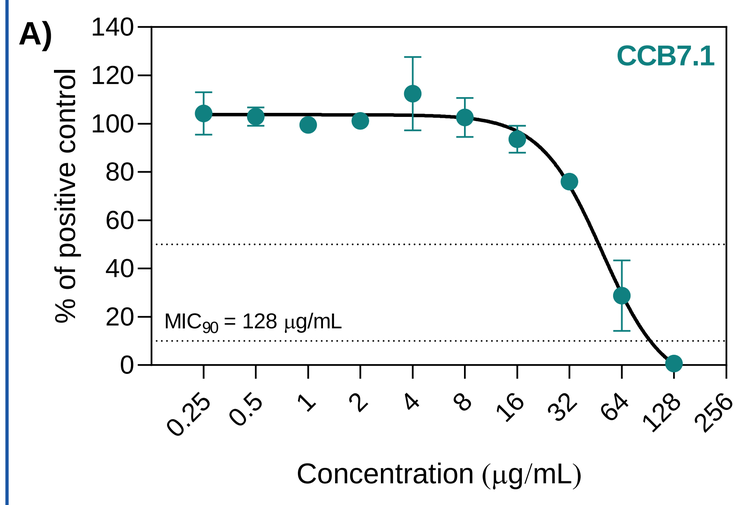
- ✓ We have developed a reproducible, high-throughput biofilm model representative of BV.
- ✓ Our model is characteristic of the clinical dysbiotic BV biofilm, compositionally dominated with *G. vaginalis*, supplemented with accessory pathogens *F. vaginae, M. curtisii* and *P. bivia*.
- ✓ BV biofilm model can tolerate increased concentrations of metronidazole and clindamycin but is susceptible to Lactobacilli-based probiotic therapy.
- ✓ *Gardnerella* specific endolysins demonstrate some significant activity against *G. vaginalis* within our model and can alter the overall community dynamic and composition.

References

1. Ellington, K., and Saccomano, S, K., (2020). *Recurrent bacterial vaginosis*. The Nurse Practitioner.
2. Arroyo-Moreno, S. *et al.,* (2022). *Identification and characterisation of novel endolysins targeting Gardnerella vaginalis biofilms to treat bacterial vaginosis*. NPJ biofilms and microbiomes.
3. Landlinger, C. *et al.,* (2021). *Engineered phage endolysin eliminates Gardnerella biofilm without damaging beneficial bacteria in bacterial vaginosis ex vivo.* MDPI Pathogens.
4. Castro, J. *et al.,* (2019). *Unveiling the role of Gardnerella vaginalis in polymicrobial bacterial vaginosis biofilms: The impact of other vaginal pathogens living as neighbours*. The ISME Journal.
5. Zozaya-Hinchliffe, M. *et al.,* (2010). *Quantitative PCR assessments of bacterial species in women with and without bacterial vaginosis.* Journal of Clinical Microbiology.

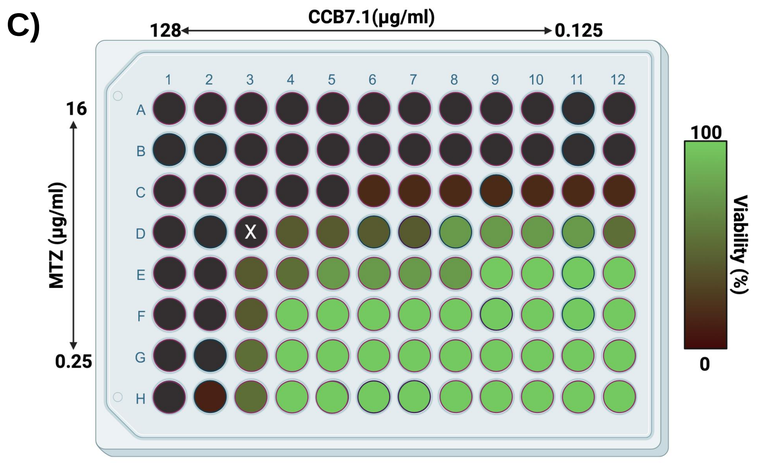
Results

Figure 4: Characterising polymicrobial BV-associated biofilm model.



B)

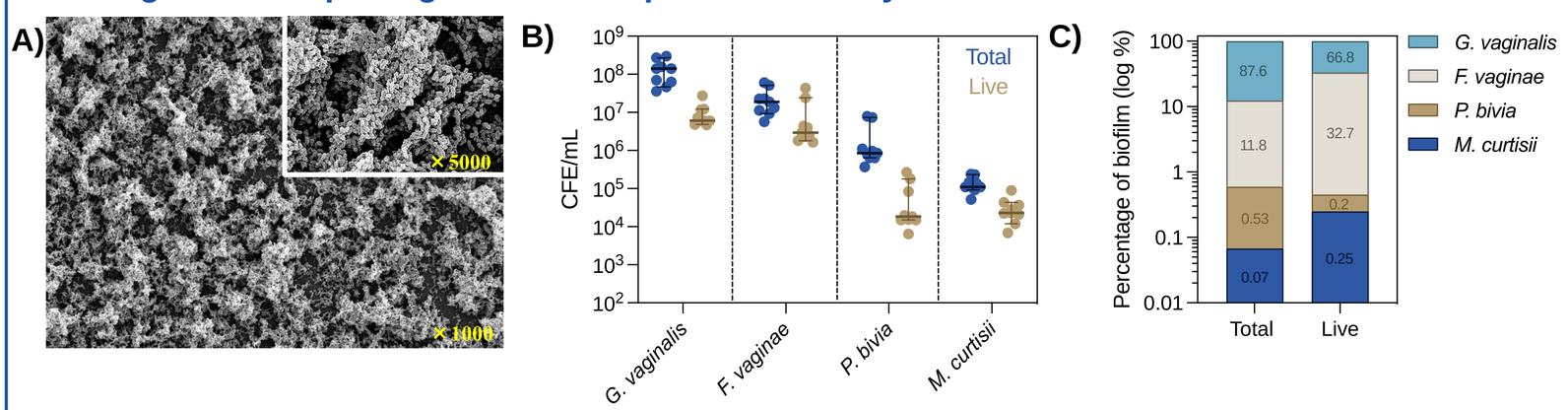
CCB7.1 Minimum Inhibitory Concentrations	
Strain	**pMIC$_{90}$ (µg/mL)**
***Gardnerella vaginalis* ATCC 14018**	**128**
***Gardnerella vaginalis* UG 860107**	**64**
***Gardnerella swidsinkii* CCUG 72429T**	**32**
***Gardnerella piotii* CCUG 72425T**	**64**
***Lactobacillus crispatus* DSM 20584**	**>256**
***Lactobacillus jensenii* DSM 20557**	**>256**



FIC index = 4 (Additive)

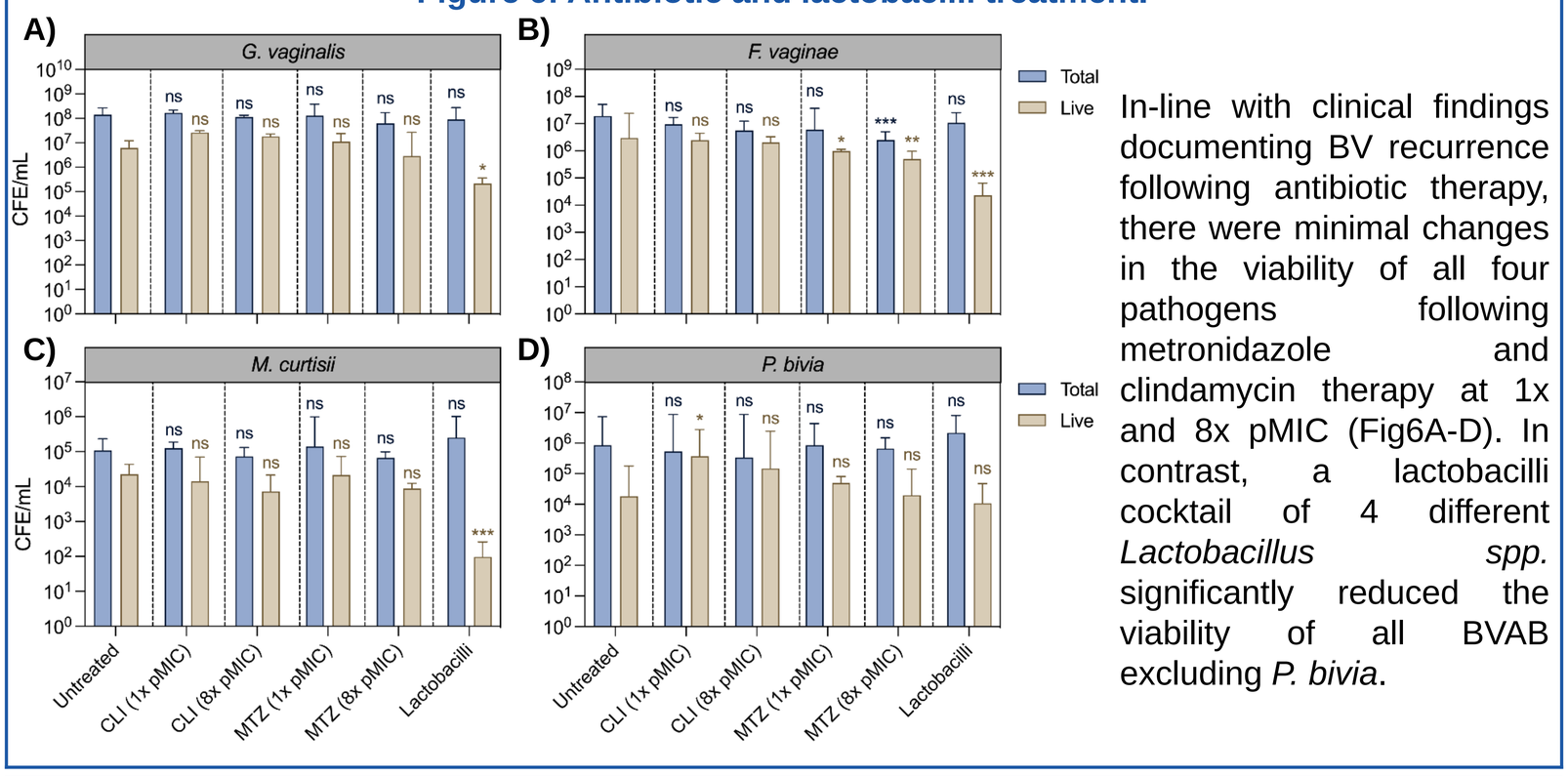
CCB7.1 was shown to be active against *G. vaginalis, G. piotii* and *G. swidsinskii*, with no activity against beneficial *Lactobacillus spp.* (Fig4A,B). Additionally, it was shown to have an additive effect when tested in combination with metronidazole (Fig4C).

Figure 5: Morphological and compositional analysis of BV-associated biofilm model.



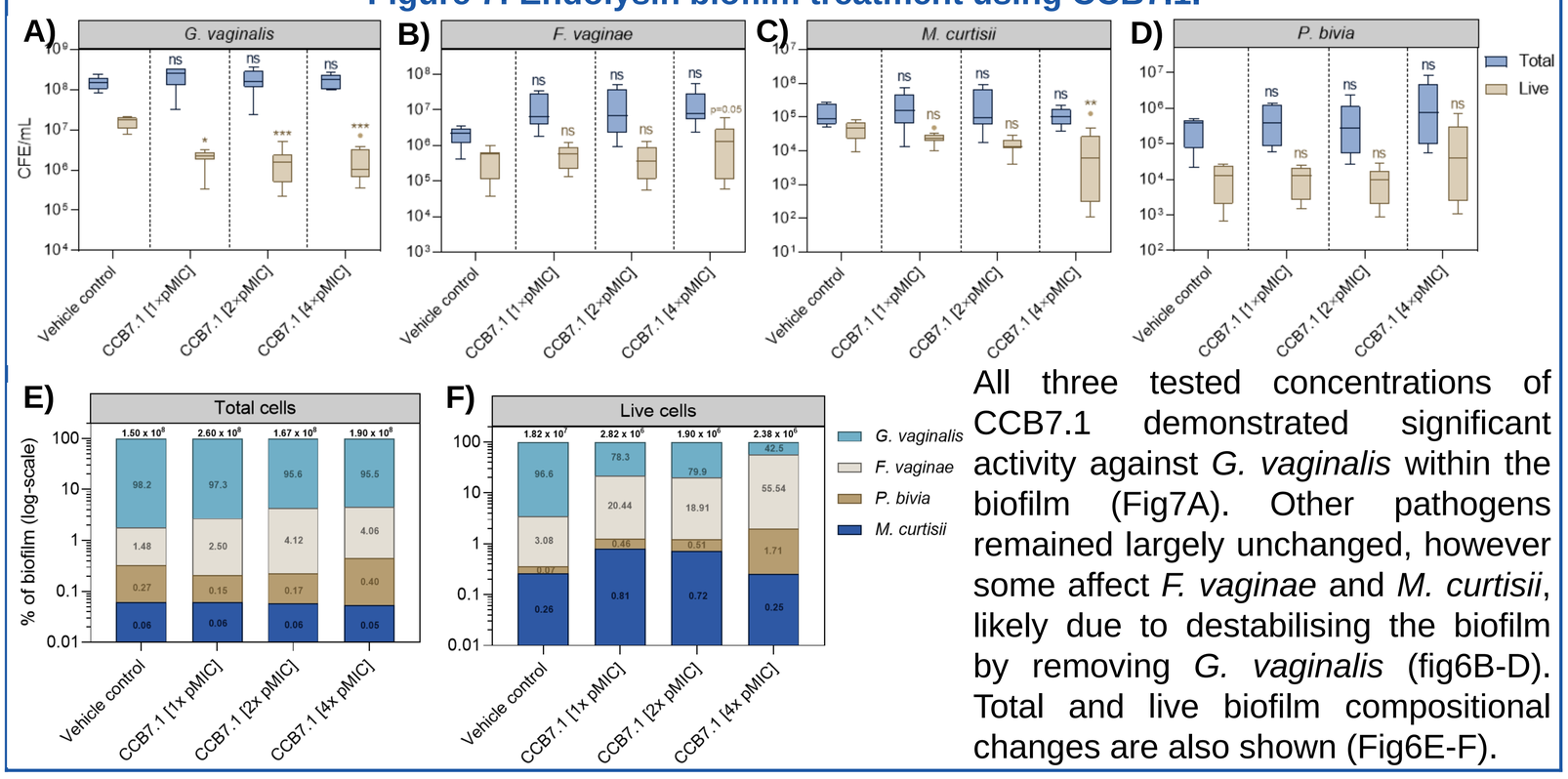
SEM displaying dense, three-dimensional biofilm architecture (Fig5A). Compositional analysis shows that all four species colonised biofilms to varying degrees (Fig5B,C). *G. vaginalis* was the most abundant (66.8% of live cells), followed by *F. vaginae* (32.7%), *P. bivia* (0.2%) and *M. curtisii* (0.25%).

Figure 6: Antibiotic and lactobacilli treatment.



In-line with clinical findings documenting BV recurrence following antibiotic therapy, there were minimal changes in the viability of all four pathogens following metronidazole and clindamycin therapy at 1x and 8x pMIC (Fig6A-D). In contrast, a lactobacilli cocktail of 4 different *Lactobacillus spp.* significantly reduced the viability of all BVAB excluding *P. bivia*.

Figure 7: Endolysin biofilm treatment using CCB7.1.



All three tested concentrations of CCB7.1 demonstrated significant activity against *G. vaginalis* within the biofilm (Fig7A). Other pathogens remained largely unchanged, however some affect *F. vaginae* and *M. curtisii*, likely due to destabilising the biofilm by removing *G. vaginalis* (fig6B-D). Total and live biofilm compositional changes are also shown (Fig6E-F).

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