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Sessile *Clostridioides difficile* contribute towards recurrent *C. difficile* infection

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Background

C. difficile infection (CDI) is a significant cause of patient morbidity and cost to healthcare providers, with 20-30%[1] of these cases recurring after primary antibiotic treatment. Most recurrent cases are due to the original strain, suggesting a protective niche that can play a role in long-term persistence/colonisation.

C. difficile forms a biofilm *in vitro* and *in vivo*, interacting with other intestinal microbes but the contribution of these biofilm-dwelling *C. difficile* cells towards recurrent infections is unknown.

Using a *in vitro* model adapted for biofilm sampling, we investigated the role of sessile *C. difficile* in recurrent CDI and how other sessile microbiota interact with *C. difficile*.

Methods

1. *In vitro* gut model

- Each gut model consists of three chemostat vessels arranged in a weir cascade fashion, replicating the physiological conditions of the proximal to distal colon.
- After the addition of *C. difficile* spores, CDI was induced with clindamycin and treated with vancomycin.

2. Biofilm donor/recipient gut model

- Biofilm communities residing on support structures were transferred from a Donor model to a Recipient model as described in **Fig1A**.

3. Polymicrobial biofilms

- Microbial cultures were diluted (1:10) in pre-reduced BHIS+C in 24-well plates.
- Biofilms were grown for 3 days and biomass measured by crystal violet and enumerated by agar culturing.

Results – Sessile *C. difficile* causes disease

- *C. difficile* became associated with the sessile populations upon addition into the model, and clindamycin induction resulted in increased vegetative cells and toxin production **(Fig1A/B).** Vancomycin reduced the planktonic *C. difficile* levels to below the limit of detection; however; sessile *C. difficile* remained unaffected **(Fig1C).**
- Approximately 4.1 $\log_{10}$ cfu/g biomass of sessile *C. difficile* cells (**Fig1C**) were transferred into a *C. difficile* naïve model, which had been previously treated with clindamycin to create an environment conducive for CDI.
- At 9 days post transfer, planktonic vegetative *C. difficile* cells were detected followed by the production of toxin.

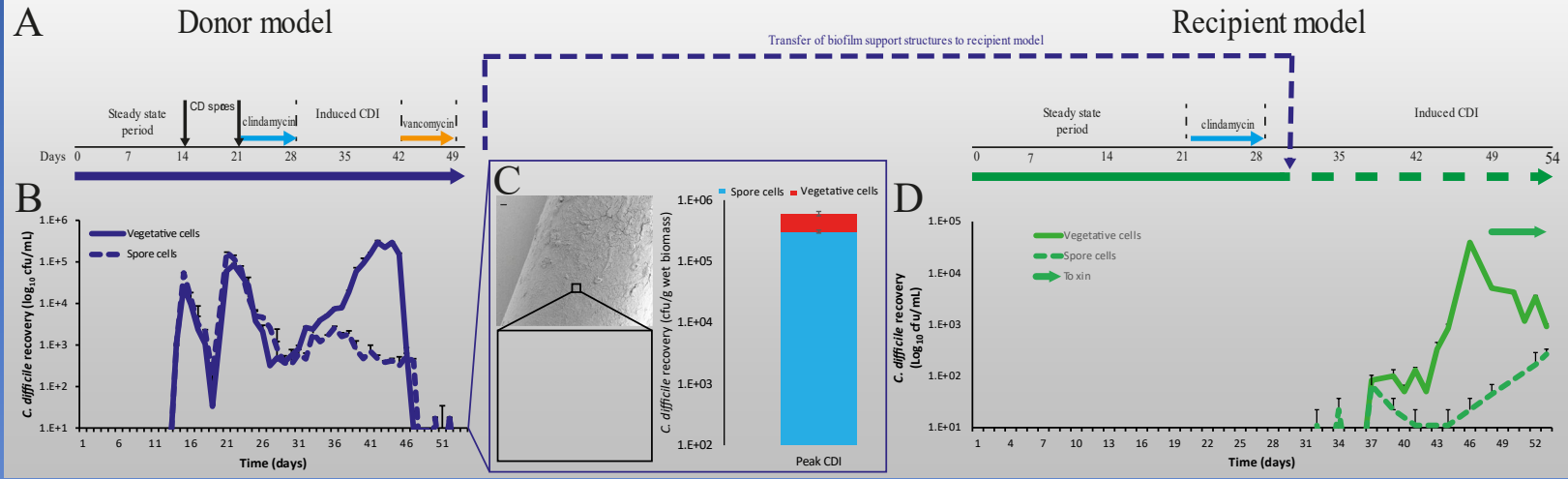

Figure 1. Transfer of sessile communities harbouring *C. difficile* from donor model into a naïve model causes CDI (A). Planktonic *C. difficile* levels during CDI (B) and levels of sessile *C. difficile* on each support structure (C). Planktonic *C. difficile* levels in the recipient model (D). Biofilm transfer occurred on day 31.

Results – Sessile microbiota can affect *C. difficile* biofilm formation

- 6 bacteria had an antagonistic effect, and 6 microbes had a synergistic effect on *C. difficile* biofilm formation.
- The antagonistic effect of *L. rhamnosus* and *B. longum* caused a real-term reduction in *C. difficile* cells in the biofilm, and an additive effect on *C. difficile* biofilm formation; a reduction of 4.4 $\log_{10}$ cfu/mL was seen compared with mono-biofilm (data not shown).

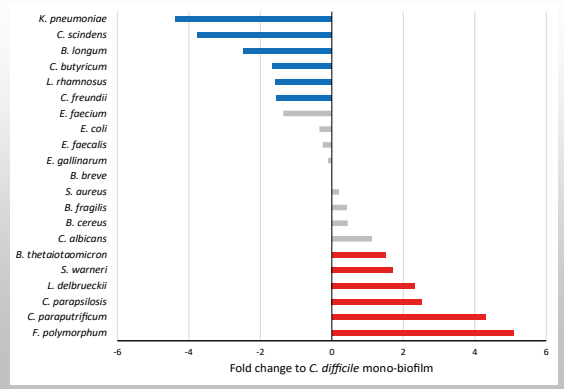

Figure 2. Antagonism (blue bars) and synergism (red bars) of sessile microbes on *C. difficile* biofilm formation. Dual cultures of *C. difficile*/sessile microbes were compared with *C. difficile* mono-biofilms.

Conclusions

- *C. difficile* was able to associate with the sessile community where vancomycin CDI therapy did not affect these populations.
- Specifically, sessile *C. difficile* cells were able to cause CDI suggesting a role for these populations in rCDI.
- The interaction between sessile microbes can either inhibit or promote *C. difficile* biofilm formation.
- Biofilm inhibition by *L. rhamnosus* and *B. longum* reduced the number of *C. difficile* cells within the biofilm.
- Future therapies need to consider this important reservoir to prevent rCDI.