Validation of a triple stage *in vitro* human gut model to study the biofilm mode of growth of *C. difficile* and the indigenous gut microbiota

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Background

- Human gastrointestinal tract harbours complex microbial communities – planktonic and biofilm
- **Biofilm** - aggregation of bacteria bound to a substratum material and encased in a self produced extra-cellular matrix
- Bacteria within biofilms differ from planktonic bacteria:
- C. *difficile* adheres to colonic cells and mucus*
- Little else known about presence of CD in biofilms

*Gomez-Trevino, M *et al* 1996; Karjalainen, T *et al* 1994

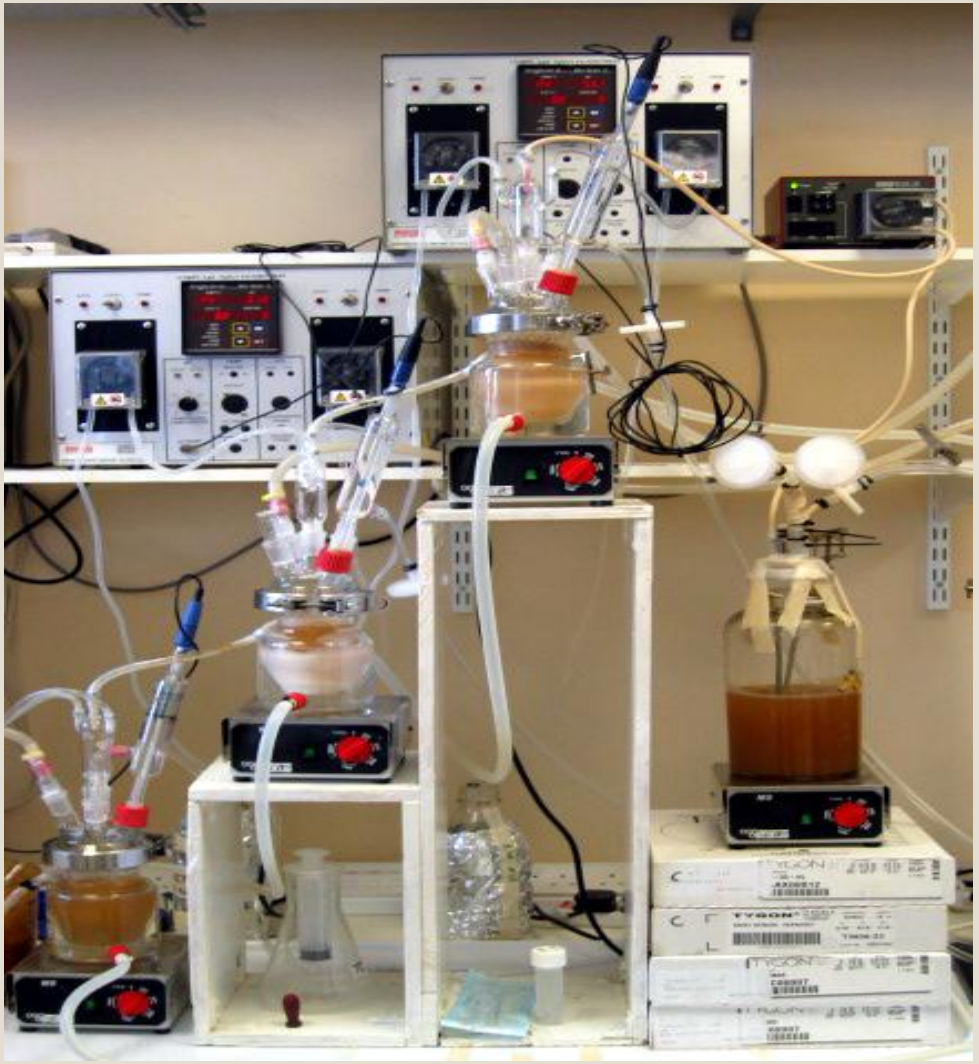
In vitro human gut model

- Successfully validated and well established model in which to study CDI

- Successfully used to:
  - compare the efficacies of therapies
  - induce CDI
  - study effect of antimicrobial administration on the human gut microbiota

In vitro human gut model

- Complex growth medium
- Pooled human faecal emulsion
- Anaerobic
- 37 ° C
- pH 5.5 – 6.8
- Planktonic indigenous gut microbiota and CD populations monitored



Proximal colon

Distal colon

In vitro human gut model

- Biofilm present on walls of vessel within the original gut model
- Spikes in planktonic bacterial populations
- Biofilm shedding?
- CD present?
- Treatment failure/recurrence?

In vitro human gut model

- Sampled biofilm at end of experimental run and compared to planktonic:
  - CD germination, proliferation, toxin production and treatment

	Toxin
Planktonic	✗
Biofilm	✓

- Cytotoxin persistence?
- CD toxin producing strain present within biofilm?

Baines *et al* Unpublished data

PhD

Aims

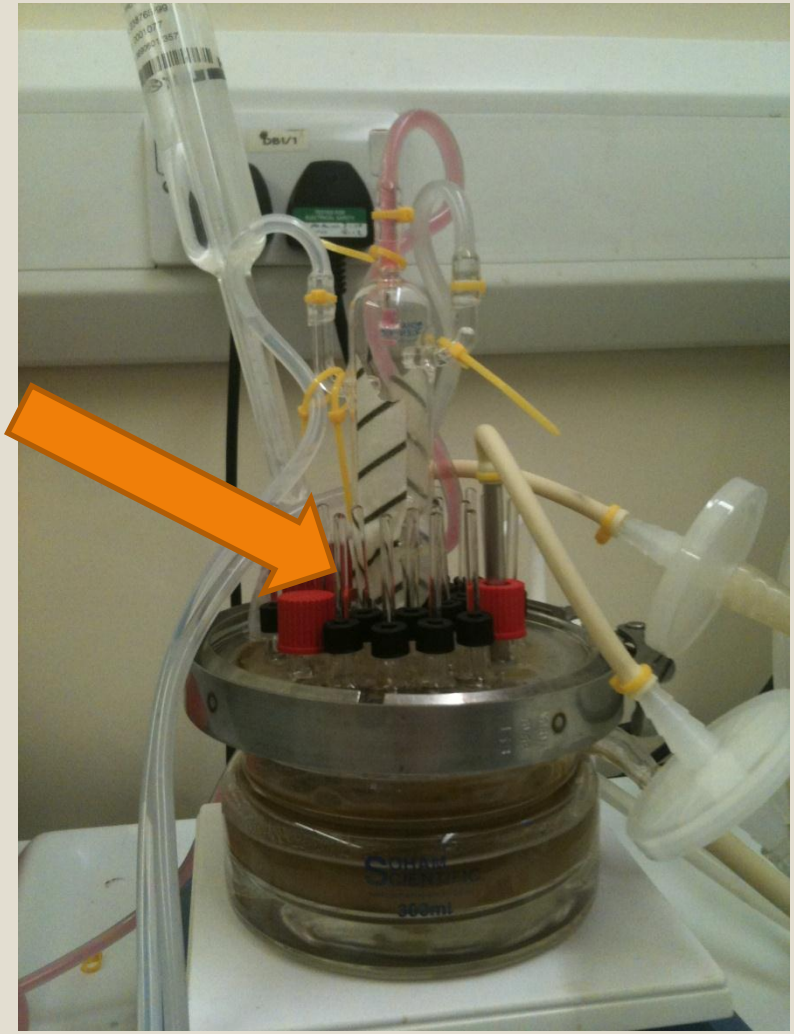
- Adapt human gut model to study planktonic and sessile bacterial populations
- Facilitate the formation of biofilm
- Easily sample sessile and planktonic populations longitudinally
- Evaluate role of biofilms in treatment failure/recurrence of CDI

Investigational/preliminary work

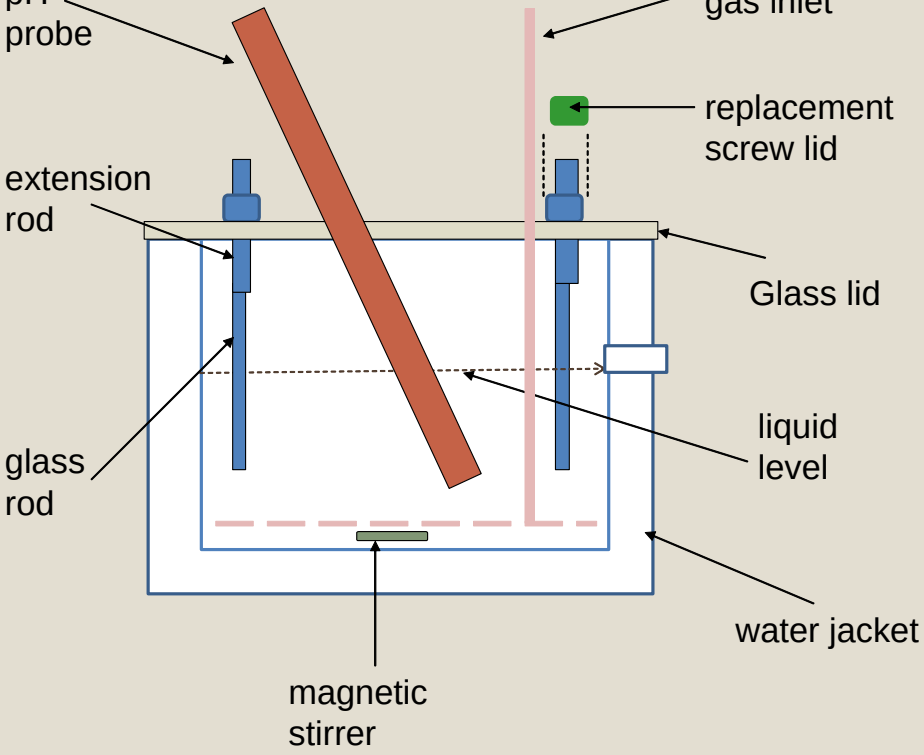
- **Biofilm scraping**
  - Mature biofilm, difficult to sample
- **Mucin alginate beads**
  - Realistic substratum material
  - Degradation
- **Glass beads**
  - Difficulty sampling
- **Glass rods**
  - Increased biofilm on liquid/air interface

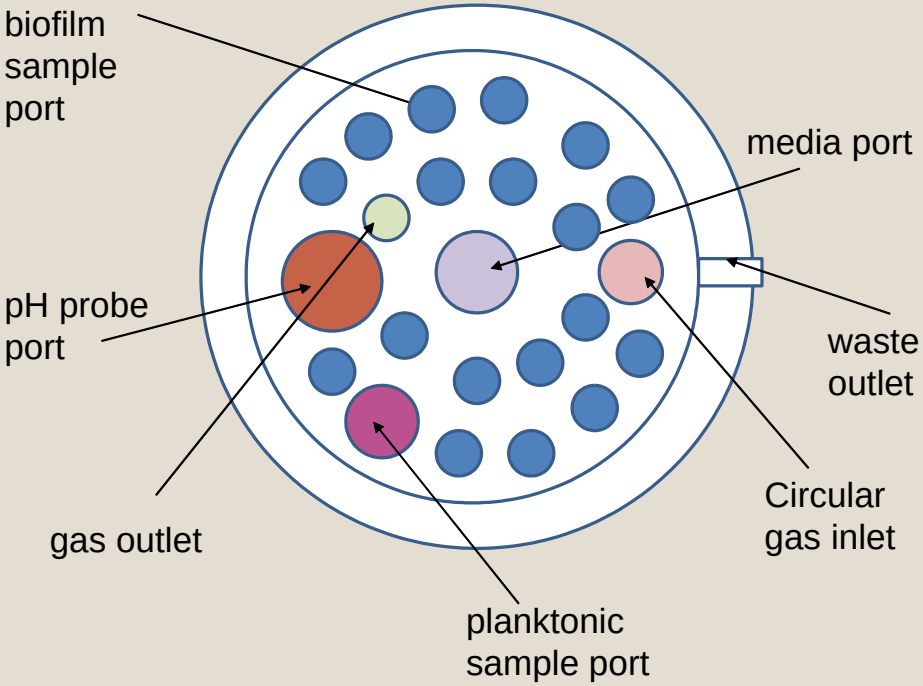
Biofilm human gut model

- Adapt original gut model
- Maintain basic vessel design
- Maximise number of rods/sample points
- Ensure biofilm can be sampled with ease



Biofilm human gut model



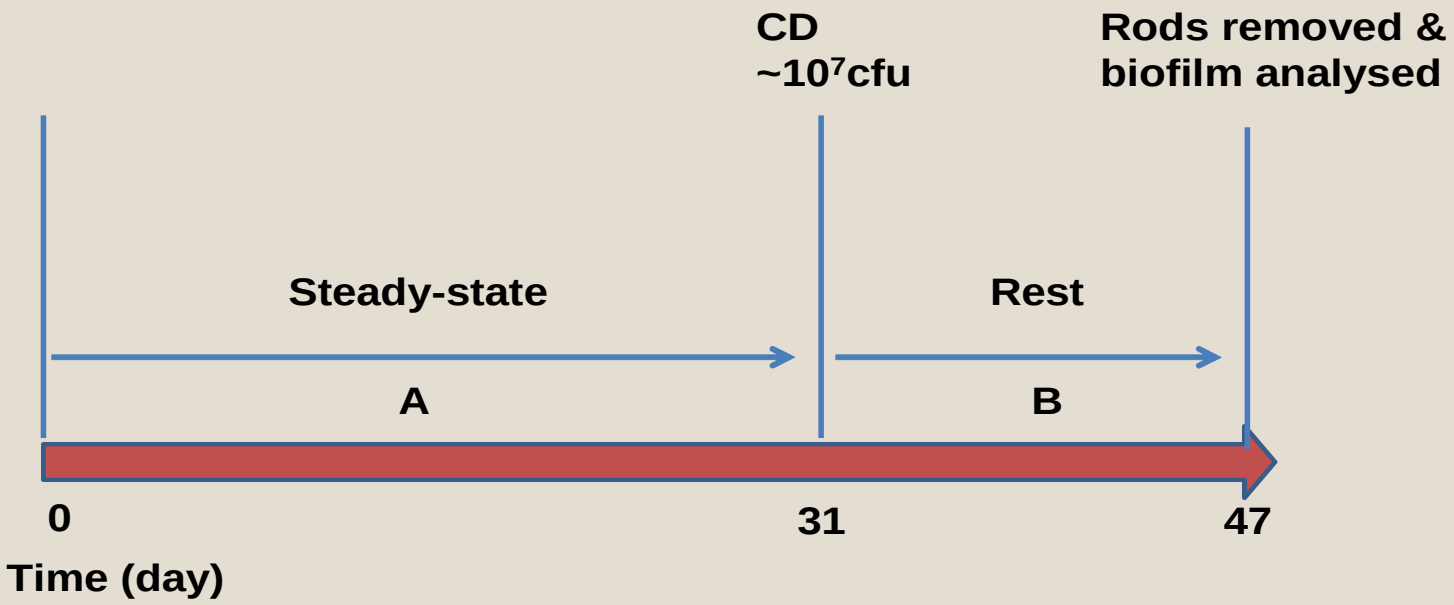


Biofilm human gut model

- Validation of a human gut model to facilitate and monitor the formation of biofilm
  - Ensure biofilm forms on all rods
  - Ensure consistency of biofilm populations across all rods
  - Ensure reproducibility of model
  - Ensure that planktonic populations behave as they did in original gut model

Biofilm human gut model - methods

- Single stage model
- 18 rods



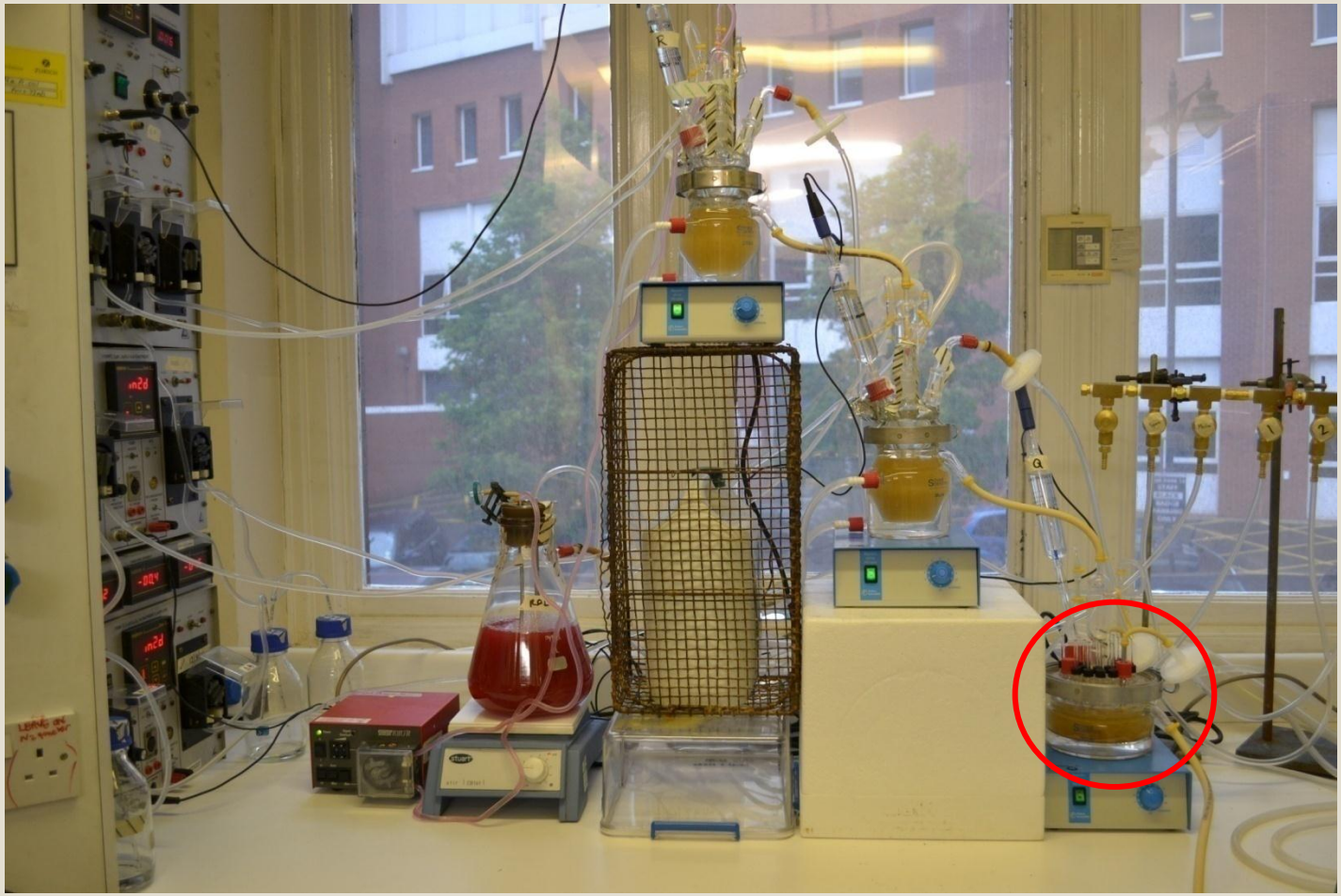
- Monitor planktonic populations throughout
- Experiment repeated on separate occasion

Biofilm human gut model - results

Results

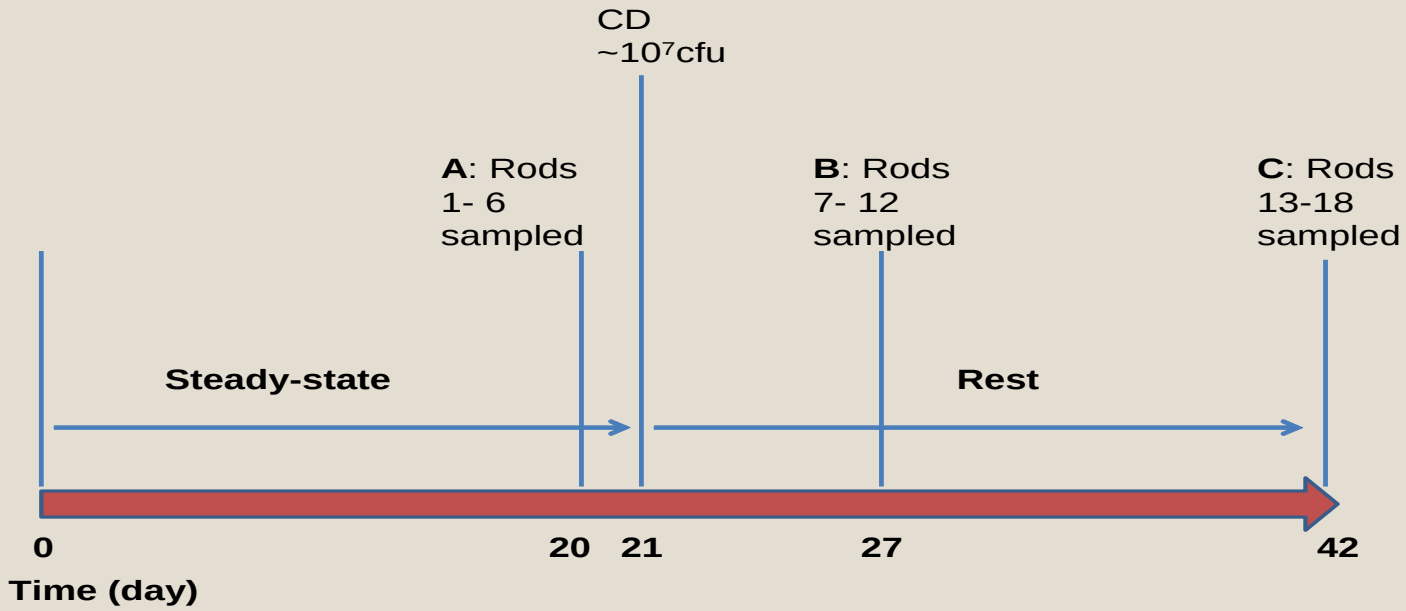
- All glass rods facilitated biofilm formation
- Planktonic and sessile populations were comparable including C. *difficile*
- Variation of total biofilm biomass amongst the rods
- Populations of each bacterial group in $\log_{10}$cfu/g had minimal variation between rods
- Repeat experiment had similar results

Triple stage biofilm human gut model



Triple stage biofilm human gut model

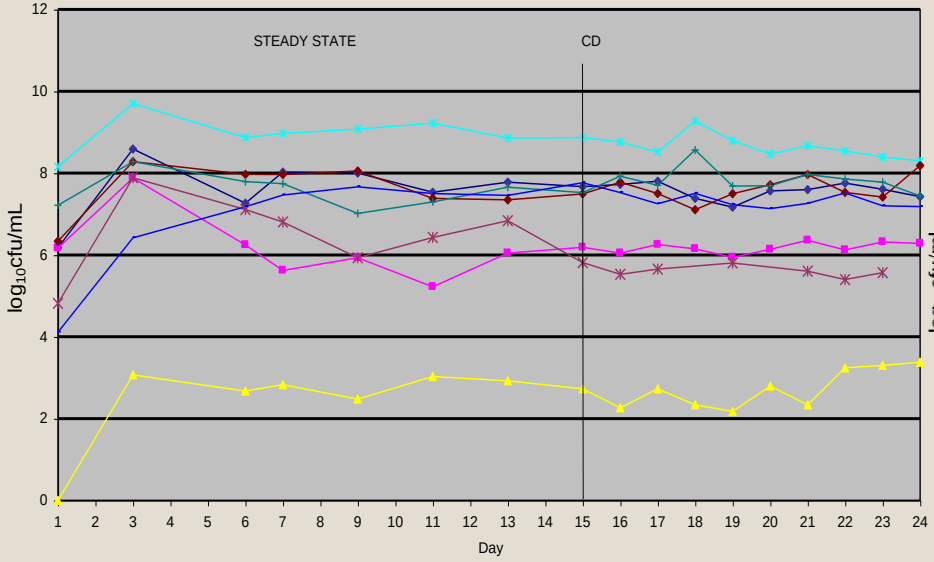
- Vessel design same as in single stage



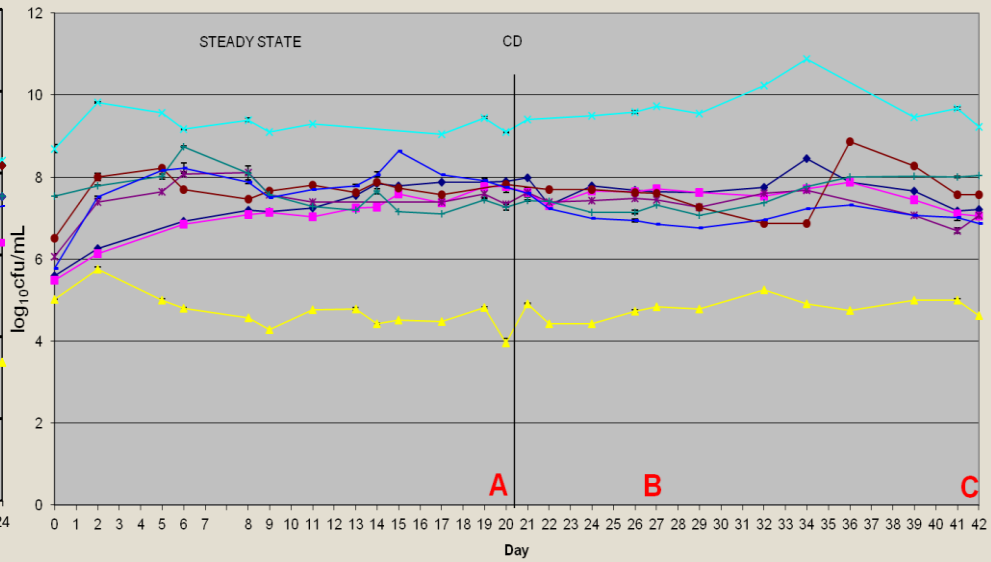
- Six rods were sampled at three different time points (A, B and C)
- Planktonic populations monitored throughout
- Experimental design repeated on separate occasion

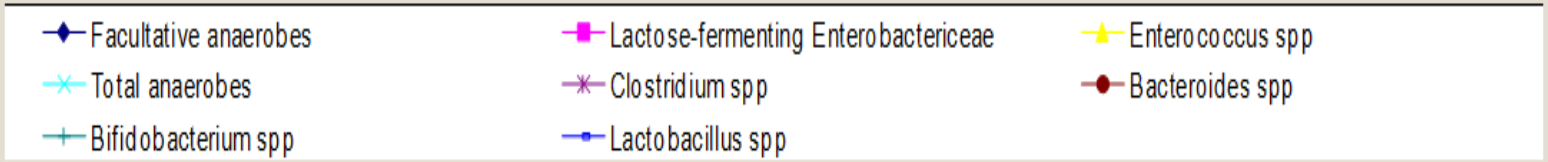
Triple stage biofilm human gut model

Planktonic populations of indigenous gut microbiota in vessel 3 of the **original** human gut model



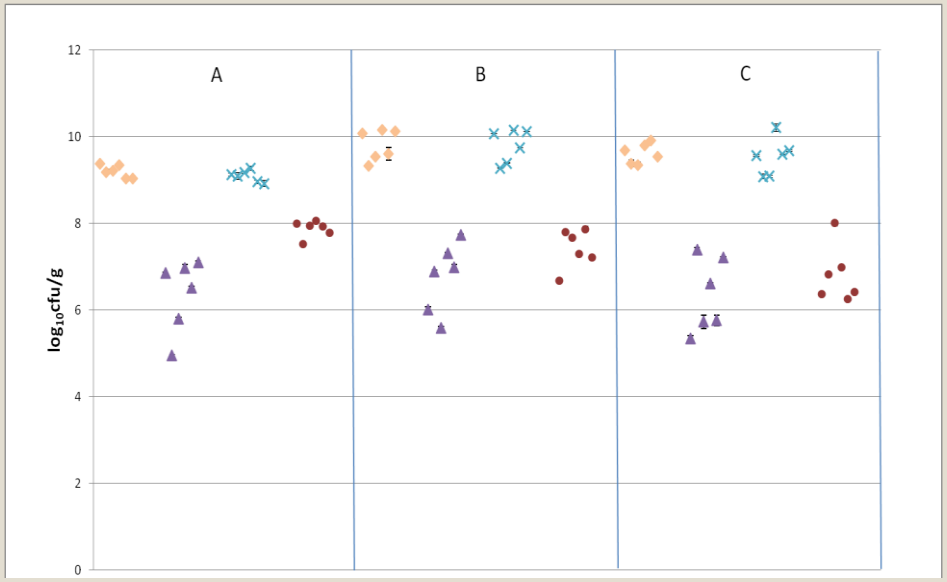
Planktonic populations of indigenous gut microbiota in vessel 3 of the **biofilm** human gut model



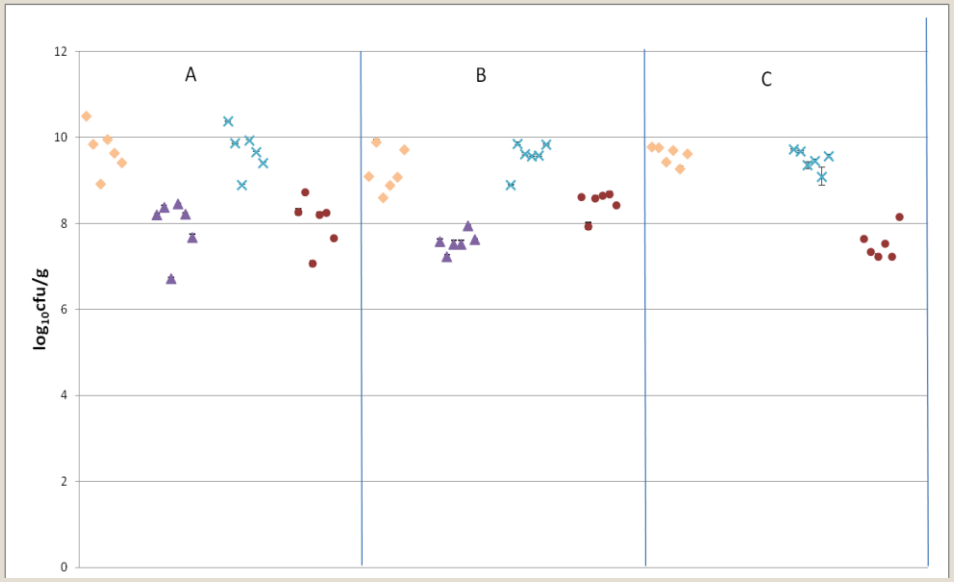


Triple stage biofilm human gut model

Sessile populations of facultative anaerobes within the triple stage biofilm gut model # 1

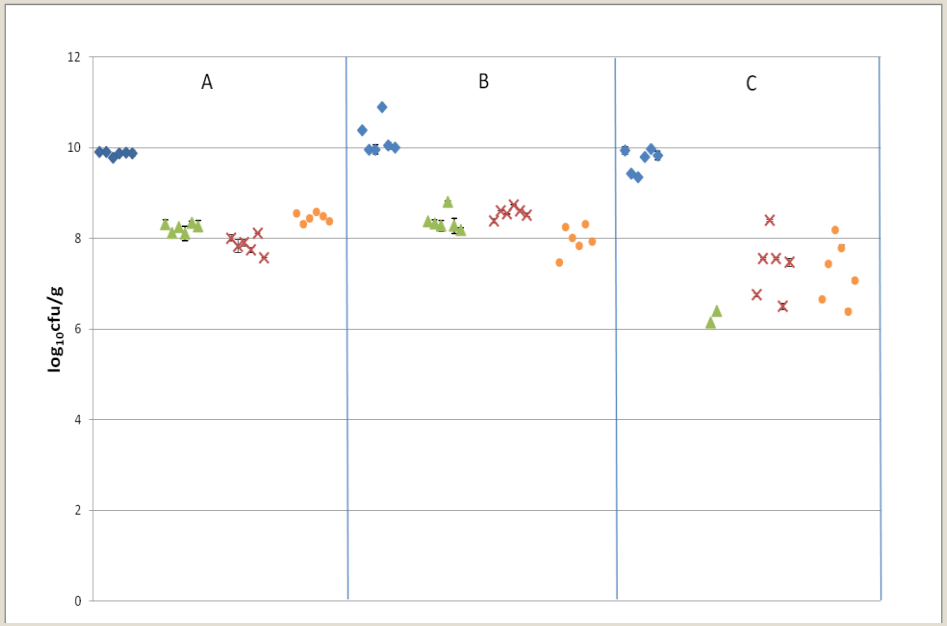


Sessile populations of facultative anaerobes within the triple stage biofilm gut model # 2

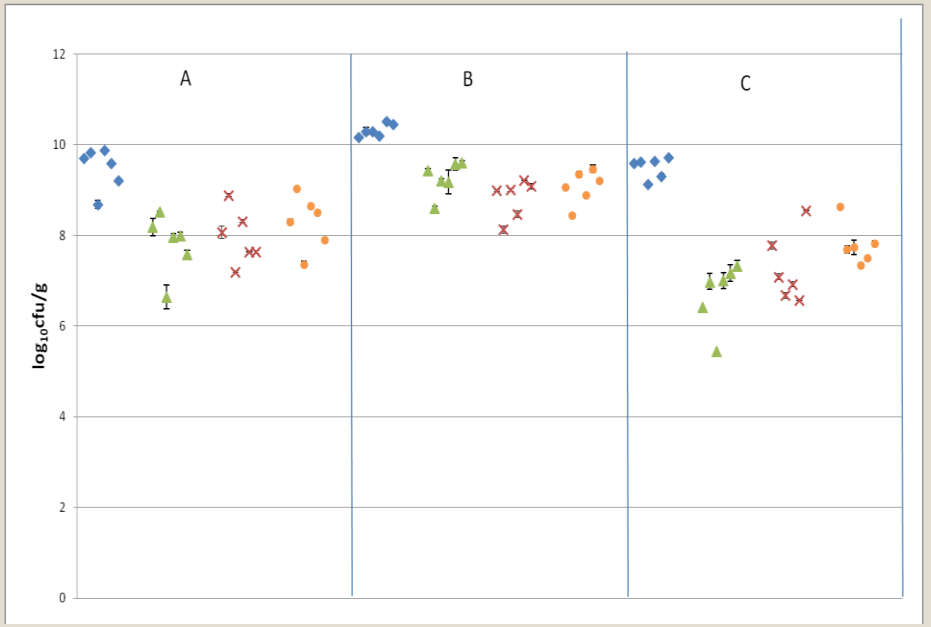


Triple stage biofilm human gut model

Sessile populations of obligate anaerobes within the triple stage biofilm gut model # 1

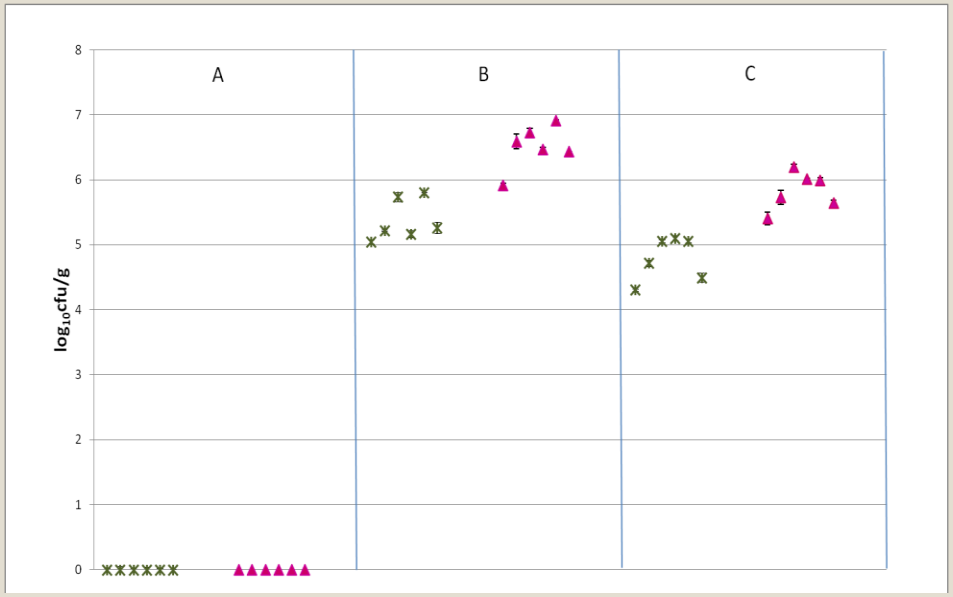


Sessile populations of obligate anaerobes within the triple stage biofilm gut model # 2

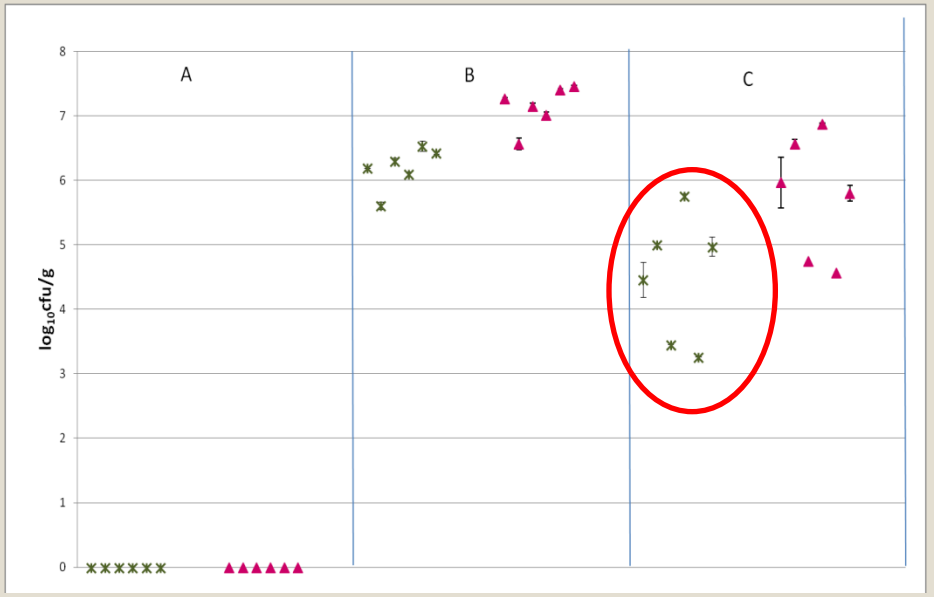


Triple stage biofilm human gut model

Sessile populations of C. *difficile* within the triple stage biofilm gut model # 1

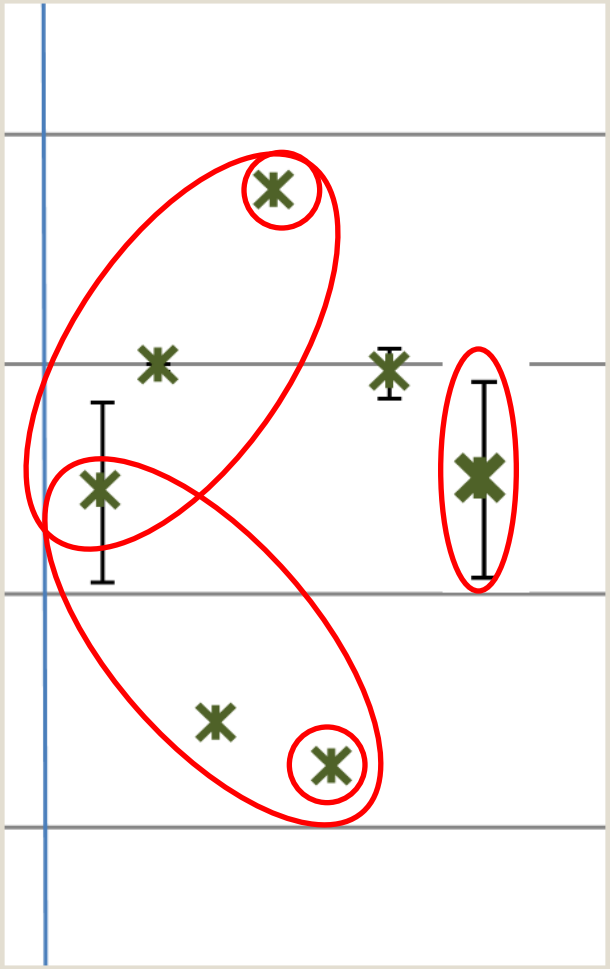


Sessile populations of C. *difficile* within the triple stage biofilm gut model # 2



C. diff TVC C. diff spo

Triple stage biofilm human gut model



Triple stage biofilm human gut model

Conclusions

- Planktonic populations similar to original gut model
- All bacterial groups present within biofilm including *C. difficile* spores
- Biofilm forms consistently on all rods
- Total biofilm biomass is variable
- Biofilm formation has minimal variation between rods
- The biofilm model is reproducible
- Limitation – finite sampling points

Triple stage biofilm gut model has been successfully validated for the study of indigenous gut microbiota biofilms

Future ……

- Validate the biofilm human gut model for studying the role of biofilms in CDI
  - Induce CDI
  - Treatment of CDI
  - Post treatment
  - **Does *C. difficile* persist within biofilms after treatment? Treatment failure/recurrence?**

- Evaluate drugs for their capacity to treat biofilm infections *in vitro*

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