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Efficacy of alternative fidaxomicin dosing regimens for treatment of simulated *Clostridium difficile* Infection in an *in vitro* human gut model

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Synopsis

Background: Fidaxomicin treatment reduces risk of recurrent *Clostridium difficile* infection (CDI) compared with vancomycin. Extending duration of fidaxomicin therapy may further reduce recurrence. We compared the efficacy of four extended fidaxomicin regimens in an *in-vitro* model of CDI.

Methods: Four gut models were primed with human faeces, spiked with *C. difficile* spores (PCR ribotype 027) and clindamycin instilled (33.9 mg/L, four times daily, seven days) to induce simulated CDI. Four extended fidaxomicin treatment regimens were evaluated: model 1, 20 days, 200mg/L twice daily; model 2, 5 days 200mg/L twice daily, 5 days rest, 5 days 200mg/L twice daily; model 3, 5 days 200mg/L twice daily, 5 days rest, 10 days 200mg/L once daily; model 4, 5 days 200mg/L twice daily, 20 days 200mg/L once every other day. *C. difficile* populations, toxin, gut microbiota and antimicrobial levels were monitored daily.

Results: All fidaxomicin regimens successfully resolved simulated CDI without recurrence. Five days of fidaxomicin instillation was barely sufficient to resolve CDI (models 2, 3 and 4). A second pulse or tapered dosing further reduced *C. difficile* and toxin detection. All regimens were sparing of microbiota, affecting only enterococci and bifidobacteria. Pulsed or tapered regimens allowed greater bifidobacteria recovery than the extended (20 day) regimen. Bioactive fidaxomicin persisted throughout the experiment in all models at concentrations inhibitory to *C. difficile.*

Conclusions: Pulsed or tapered fidaxomicin regimens may enhance suppression of *C. difficile* whilst allowing microbiota recovery; clinical studies are required to ascertain the potential of this approach in further reducing recurrent CDI.

Introduction

As the leading cause of infective antibiotic-associated diarrhoea and colitis,[1] *Clostridium difficile* infection (CDI) continues to place a significant burden on healthcare facilities worldwide.[2, 3] Much of the burden is due to high rates of CDI recurrence (20-30%),[4, 5] which lead to increased duration of treatment and hospital stay or readmission. Rates of recurrence have increased since the 1980s, coincident with the emergence of epidemic strains.[6] During randomised controlled trials comparing fidaxomicin with vancomycin for the treatment of CDI, recurrence rates in patients infected by PCR ribotype 027 strains were significantly higher than those associated with other strains.[7] Current guidelines generally recommend oral metronidazole and oral vancomycin for treatment of mild to

moderate and severe CDI, respectively.[8] However, treatment failure and high rates of recurrence have been reported for both treatment agents.[9, 10] Patients experiencing one recurrence are significantly more likely to experience further recurrences.[11] Current guidelines suggest that first recurrences should be treated in the same way as initial CDI, but taking severity of disease into consideration.[8] For second and subsequent recurrences, prolonged pulsed and/or tapered vancomycin is sometimes used,[8] but with no clear preference for a particular regimen.

Fidaxomicin, a narrow-spectrum macrocyclic antimicrobial, has recently been approved for treatment of CDI. In two phase III randomised, double-blind clinical trials, 200mg twice daily fidaxomicin demonstrated non-inferiority to 125mg four times daily vancomycin for initial clinical cure of CDI, but was superior to vancomycin in prevention of recurrence, and so for sustained clinical cure.[12] It is possible that different dosing regimens of fidaxomicin may be beneficial in further reducing CDI recurrence. We have previously described the efficacy of fidaxomicin for treatment of simulated CDI in a validated human gut model.[13] Here we report the efficacy of four dosing regimens to investigate the affects of extended, pulsed and tapered fidaxomicin on CDI resolution using the same *in vitro* gut model.

Methods

Triple-stage chemostat gut model.

The gut model used in this experiment was based on that of MacFarlane *et al*[14] and comprises three glass vessels arranged in a weir cascade formation. The model is inoculated with a pooled faecal emulsion from healthy volunteers over 60 years of age (n=5), and top-fed with a complex growth medium (dilution rate, 13.2 mL/h).[15] The vessels are maintained at 37°C and pH 5.5, 6.2 and 6.8 for vessels 1, 2 and 3, respectively. All vessels are sparged with oxygen-free nitrogen to maintain an anaerobic environment. The system has been validated against the intestinal contents of sudden death victims,[14] and provides a close simulation of bacterial activities and composition in different areas of the colon.

C. difficile strains

C. difficile strain 027 210 (BI/NAP1/PCR ribotype 027/ toxinotype III) was used in all experiments. The strain was originally isolated during an outbreak of CDI at the Maine Medical Centre (Portland, ME, USA) in 2005, and was kindly supplied by Dr Robert Owens (formerly, Maine Medical Centre).

Experimental design

Four gut models were run in total (Figure 1). All models were inoculated with ~150 mL 10% (w/v) faecal slurry prepared from pooled, *C. difficile*-negative faeces and left to equilibrate for 14-21 days (Period A) to allow bacterial populations to achieve steady state. A single aliquot of *C. difficile* PCR ribotype 027 spores (~10^7 cfu) was added into vessel 1 of each model, and left for a control period of 7 days (Period B), before a second aliquot of *C. difficile* spores was added, and clindamycin instillation commenced to induce simulated CDI (33.9 mg/L , four times daily, 7 days, Period C). Once germination, vegetative *C. difficile* proliferation (as measured by an increase in total viable counts compared to spore counts), and high level toxin production was observed, simulated CDI was deemed to be present. Fidaxomicin treatment regimens began the day after high level toxin was observed, as outlined below:

model 1 (extended dosing): clindamycin induction of CDI (Periods C and D), followed by fidaxomicin extended dosing (200mg/L, twice daily, 20 days, Period E);

model 2 (pulsed dosing): clindamycin induction of CDI (Periods C and D), followed by fidaxomicin pulsed dosing comprising five days initial pulse of antibiotic instillation (200 mg/L twice daily, 5 days, Period E), five days rest (Period F), and a further pulse of five days fidaxomicin. (200 mg/L twice daily, 5 days, Period G);

model 3 (pulsed-tapered dosing): clindamycin induction of CDI (Periods C and D) followed by fidaxomicin pulsed-tapered dosing comprising five days initial pulse of fidaxomicin (200 mg/L twice daily, 5 days, Period E), five days rest (Period F), and a further tapered fidaxomicin instillation period (200 mg/L once daily, 10 days, Period G);

model 4 (tapered-pulsed dosing): clindamycin induction of CDI (Periods C and D) followed by a fidaxomicin tapered-pulsed dosing regimen comprising five days of fidaxomicin (200 mg/L twice daily, 5 days, Period E), immediately followed by further 20 day tapered-pulsed fidaxomicin instillation period (200 mg/L once every other day, 20 days, Period F).

All models were monitored for a further three weeks after treatment cessation with no further interventions. Recurrence of simulated CDI was defined as a recurrence of vegetative *C. difficile* proliferation (as measured by an increase in total viable counts compared to spore counts)and associated toxin production.

Gut microbiota populations were enumerated on selective and non-selective agars every other day during Period A, and daily thereafter, as recently described in detail.[16] *C. difficile* total viable counts

(vegetative cells plus spores), spore counts and toxin production were monitored daily as previously described[16] from Period B onwards, and daily antimicrobial concentration was measured by large plate bioassay from Period C onwards.

Monitoring for emergence of isolates of C. difficile with reduced susceptibility to fidaxomicin.

The MIC of fidaxomicin for the *C. difficile* 027 210 strain used in these gut model experiments was 0.25 mg/L (by agar incorporation testing). Reduced susceptibility of *C. diffcile* to fidaxomicin was monitored on Brazier's CCEYL containing four times the MIC (i.e. 1 mg/L) fidaxomicin in addition to the usual supplements.

Determination of antimicrobial concentrations

Samples (1 mL) from all vessels of each gut model were centrifuged (16000 g) and the supernatants stored at -20˚C. Wilkins-Charlgren agar (100 mL) was sterilized by autoclaving, cooled to 50˚C, inoculated with 1mL *Kocuria rhizophila* (ATCC 9341) indicator organism suspension and transferred aseptically into 245x245 mm agar plates. Inoculated agars were dried (37˚C) for 10 min and 25 wells (9 mm diameter) were removed from the agar using a cork borer. Twenty microlitres of antibiotic calibrator or sample supernatant from the gut model were inoculated into bioassay wells. Samples assayed for clindamycin concentration (Periods C and D) were sterilized by filtration through 0.22 µm syringe filters; samples assayed for fidaxomicin were not filtered as the antibiotic can adhere to glass and plasticware.[16] Bioassay plates remained at ambient temperature for 4 h prior to overnight aerobic incubation at 37˚C. Zone diameters were measured using callipers accurate to 0.1 mm. Calibration lines were plotted from squared zone diameters and unknown concentrations from culture supernatants determined. All assays were performed in triplicate.

This study was approved by the University of Leeds, School of Medicine Research Ethics Committee (no. HSLTLM/12/061).

Results

C. difficile total viable counts, spore counts and cytotoxin

Before (Period B) and during (Period C) clindamycin instillation *C. difficile* populations remained as spores (total viable counts are equal to spore counts), and decreased from ~5-6 $\log_{10}$ cfu/mL to ~3-4 $\log_{10}$ cfu/mL as spores washed out of the model at the rate of dilution (Figure 2a-d). After clindamycin instillation, *C. difficile* spore germination and vegetative cell proliferation (an increase in

total viable counts compared to spore counts) was observed in all models. In models 1 (extended dosing), 3 (pulsed-tapered dosing) and 4 (tapered-pulsed dosing), spore germination was observed ~6-7 days after the end of clindamycin instillation (Figures 2a, 2c and 2d, Period D), whereas in model 2 (pulsed dosing), germination was not observed until 21 days after clindamycin instillation (Figure 2b, Period D). However, in all models cytotoxin was detected 1-3 days after germination, and reached a maximum titre of 3-4 relative units (RU). Fidaxomicin instillation rapidly reduced *C. difficile* total viable counts in all models (~5 $\log_{10}$ cfu/mL reduction). In model 1 (extended dosing), 20 days of fidaxomicin instillation reduced both total viable counts and spore populations to below the limit of detection for the duration of antibiotic administration (Figure 2a, Period E), and for the remainder of the experiment (Period F), with only sporadic spore detection on two occasions. In model 2 (pulsed dosing), the first five days of fidaxomicin instillation reduced total and spore counts to ~2 $\log_{10}$ cfu/mL (Figure 2b, Period E); total and spore counts remained at this level during the five days of no antimicrobial instillation (Period F). The second five days of instillation of fidaxomicin further reduced *C. difficile* populations to around the limit of detection (Period G), with only sporadic detection for the remainder of the experiment (Period H). In model 3 (pulsed-tapered dosing), the first five days of fidaxomicin instillation caused a greater initial decrease in *C. difficile* counts than seen in model 2 (pulsed dosing), with populations reduced to around the limit of detection for the five day rest period (Figure 2c, Periods F and G). Populations remained around the limit of detection throughout the 10 days of once-daily fidaxomicin instillation, with only sporadic *C. difficile* detection throughout Periods G and H. In model 4 (tapered-pulsed dosing), the initial five day pulsing of fidaxomicin reduced *C. difficile* populations to below the limit of detection; counts remained at this level during the 20 days of alternate day fidaxomicin dosing (Figure 2d, Period F). Following the end of fidaxomicin instillation (Period G), sporadic detection of *C. difficile* increased. Although sporadic *C. difficile* was detected (total counts and spore counts) at round the limit of detection, particularly in models 2 (pulsed dosing), 3 (pulsed-tapered dosing), and 4 (tapered-pulsed), no signs of recurrent *C. difficile* vegetative growth (sustained increase of total viable counts compared to spore counts) or toxin production were observed in any of the four models.

Gut microbiota viable counts

Clindamycin instillation elicited large declines in bifidobacteria populations (at least 4 $\log_{10}$ cfu/mL) in all four models (Figure 3), and smaller declines in lactobacilli (Figure 4) and clostridia (Figure 3) populations (~2 $\log_{10}$ cfu/mL). In all models, lactobacilli and clostridia populations returned to steady state levels by the end of Period D. Bifidobacteria populations recovered to steady state levels (models 1 (extended dosing) and 4 (tapered-pulsed dosing), Figure 3a and 3d), or slightly below

(model 3 (pulsed-tapered dopsing), Figure 3c). However, in model 2 (pulsed dosing) bifidobacteria populations declined below the level of detection following clindamycin instillation, and did not recover for the remainder of the experiment (Figure 3b). Fidaxomicin instillation elicited a major decline in bifidobacteria populations to below the limit of detection in all models (Figure 3) and a more modest decline in enterococci populations (3-4 $\log_{10}$ cfu/mL, Figure 4). Effects of fidaxomicin exposure on gut microbiota were similar regardless of dosing regimen; however, bifidobacteria populations in model 1 (extended dosing) did not recover following fidaxomcin instillation, whereas in models 3 (pulsed-tapered dosing) and 4 (tapered-pulsed dosing) these recovered to approximately steady state levels by the end of the experiment (Figure 3).

Reduced susceptibility

No *C. difficile* were isolated on CCEYL breakpoint agars throughout the experimental duration of all four models (data not shown).

Antimicrobial concentrations

In models 1 (extended dosing), 3 (pulsed-tapered dosing) and 4 (tapered-pulsed dosing), clindamycin concentrations peaked at 40-80 mg/L (Figure 2a, 2c and 2d, Periods C and D)and rapidly washed out of the model following the end of instillation (within three to four days). In model 2 (pulsed dosing) there was an increased accumulation of clindamycin, peaking at 137 mg/L, which did not fall to below the limit of detection until 11 days post-instillation (Figure 2b, Periods C and D).

In models 1(extended dosing), 3 (pulsed-tapered dosing) and 4 (tapered-pulsed dosing), fidaxomicin concentrations peaked at ~100 mg/L before decreasing (Figure 2a, 3c and 2d, Period E to end of experiment), whereas in model 2 (pulsed dosing), fidaxomicin concentration remained at 20-40 mg/L for the duration of the experiment (Figure 2b, Period E to end of experiment). Unlike clindamycin, fidaxomicin was not rapidly washed out of the model, but persisted and remained detectable until the end of the experiment in all four models (Figure 2). The levels of persistence varied; fidaxomicin concentrations in models 1 (extended dosing) and 2 (pulsed dosing) persisted at ~20 mg/L throughout the final 3 week rest period, whereas in models 3 and 4, antibiotic levels decreased further during the final rest period to <5 mg/L.

Discussion

Due to the high rates of recurrent disease associated with oral metronidazole and vancomycin,[4,5] alternative dosing regimens, such as prolonged or tapered vancomycin are sometimes used for patients developing second or subsequent recurrences.[8] However, these regimens typically extend the length and hence total amount of therapy given. The negative effects of vancomycin on the gut microbiota (notably *Bacteroides*)[17-19] mean that extended vancomycin regimens are likely to disrupt gut microbiota populations further and potentially also select for vancomycin-resistant enterococci.

Fidaxomicin has been linked to lower recurrence rates,[7,12] however recurrent disease can still occur following standard fidaxomicin therapy (200mg/L, twice daily). We have therefore investigated different regimens extending the 20 fidaxomicin doses over longer time frames, and compared this to increasing the total number of doses to 40.

All four fidaxomicin dosing regimens investigated in this study successfully resolved simulated CDI in a human gut model, with no signs of recurrent vegetative cell proliferation and toxin production. We have previously noted reduced detection of *C. difficile* spores following fidaxomicin treatment[13] and postulated that the 'sticky' nature of fidaxomicin[20] may cause it to adhere to spores, acting at the earliest stages of germination and hence preventing recovery on CCEYL agar. A similar rapid reduction in detected viable spores (2-3 $\log_{10}$ cfu/mL) was observed following these dosing regimens, although *C. difficile* spore recovery during treatment varied according to the dosing schedule. Twenty days of fidaxomicin instillation (model 1, extended dosing) led to the greatest impact on *C. difficile* recovery. In the other three models, the first 5 days of fidaxomicin appeared to be insufficient to totally resolve simulated CDI. Toxin persisted at a titre of 1 in models 2 (pulsed dosing) and 4 (tapered-pulsed dosing), and some evidence of continued *C. difficile* recovery was observed, most notably in model 2 (pulsed dosing). In model 2, the second 5 days of fidaxomicin instillation further reduced *C. difficile* total and spore counts and toxin detection. In models 3 (pulsed-tapered dosing) and 4 (tapered-pulsed dosing), the tapered fidaxomicin administration following the initial 5 day pulsing supressed *C. difficile* recovery, although sporadic detection was observed following cessation of instillation. Whilst suppression of *C. difficile* spore recovery has been postulated to be linked to reduced rates of infection,[13] the clinical relevance of reduced spore recovery is not clear. Therefore limited conclusions can be drawn regarding the differing levels of sporadic *C. difficile* detection following the 4 different dosing regimens described here. However, all dosing regimens were as successful as the previously described 7 days of fidaxomicin instillation in resolving simulated CDI in the human gut model, with no signs of recurrent vegetative cell proliferation and toxin production. [13]

The reasons for the delay in germination following clindamycin instillation in model 2 are unclear. In model 2 (pulsed dosing), clindamycin remained detectable for ~10 days following the end of instillation, whereas in in the other three models clindamycin was undetectable by between 3 and 7 days after clindamycin instillation. This may have supressed spore germination for longer, causing delayed germination. Germination, when it occurred, was quantitatively similar in all four models.

Fidaxomicin has been reported to be relatively sparing of the gut microbiota.[21, 22] The effects of the four extended fidaxomicin dosing regimens on gut microbiota populations were very similar, with decreases in enterococci populations observed in all models. In contrast with previous reports,[21, 22] but as has been previously observed in the gut model,[13] fidaxomicin instillation affected bifidobacteria populations. However, effects on bifidobacteria varied between models. Twenty days of fidaxomicin instillation (model 1, extended dosing) decreased bifidobacteria populations to below the limit of detection and prevented recovery. In model 2 (pulsed dosing), bifidobacteria populations did not recover following clindamycin instillation, so the effect of this fidaxomicin dosing regimen cannot be determined. Tapered fidaxomicin dosing regimens in models 3 (pulsed-tapered dosing) and 4 (tapered-pulsed dosing) initially reduced bifidobacteria populations to below the limit of detection, but these subsequently recovered and remained stable for the rest of the experiments. Variable effects of fidaxomicin exposure on bifidobacteria are likely due to different initial populations of bifidobacteria species present in the donor stool samples.[13, 21, 22] Nevertheless, the present studies provide evidence that tapering fidaxomicin exposure can suppress *C. difficile* and yet allow recovery of gut microbiota populations.

As reported previously,[13] detectable fidaxomicin persisted in the gut model following the end of instillation, and continued to be detected for the remainder of the experiments. Lower fidaxomicin levels persisted after tapered dosing regimens, which may help to explain the recovery of bifidobacteria in models 3 (pulsed-tapered dosing) and 4 (tapered-pulsed dosing). However, the persisting fidaxomicin concentrations exceeded the MIC for the *C. difficile* strain used in these models (0.25 mg/L). Persistence of fidaxomicin in stool samples has been shown during Phase I human volunteer studies,[23] and microbiota diversity studies during Phase III studies have shown that recovery of colonic microbiota begins during fidaxomicin therapy.[21] Tapered dosing regimens such as those described here, may therefore allow low level persistence of fidaxomicin for longer periods of time than standard dosing regimens, supressing recrudescence of *C. difficile* spores, but allowing recovery of gut microbiota populations. While persistence of low level fidaxomicin for days or weeks raises concerns of possible resistance selection, we found no evidence of emergence of reduced susceptibility of *C. difficile* associated with the four prolonged dosing regimens studied here.

In conclusion, extended, pulsed or tapered fidaxomicin treatment regimens are as successful as the previously evaluated dosing regimens in resolving CDI in an *in vitro* human gut model without recurrence. Although doubling the number of fidaxomicin doses caused the greatest suppression of *C. difficile* spore recovery, the clinical relevance of this remains unclear. Extending the standard 20 doses by pulsed and tapered regimens was equally as successful in resolving simulated CDI and preventing recurrence, without increased drug cost. Extended, pulsed or tapered dosing regimens may allow persistence of fidaxomicin at concentrations that are inhibitory to *C. difficile*, whilst allowing recovery of the gut microbiota. Such regimens should therefore be investigated clinically to determine if they have the potential to further reduce recurrent CDI. Initial case report data indicates that a fidaxomicin chaser or tapered dosing regimen may be effective in patients with multiple recurrences of CDI,[24] and a phase IV study comparing the efficacy of vancomycin to extended duration of fidaxomicin therapy in the clinical cure of CDI in the elderly has commenced.[25]

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Transparency declarations

In the past 2 years, CHC has received research funding from Astellas, Cubist and Da Volterra, and support to attend meetings from Astellas. GSC has received support to attend meetings from Astellas. MHW has received research funding from Actelion, Astellas, Biomerieux, Cubist, Pfizer, Summit and The Medicines Company and consultancies and/or lecture honoraria from Actelion, Astellas, Astra-Zeneca, Bayer, Cubist, Durata, J&J, Merck, Nabriva, Novacta, Novartis, Optimer, Pfizer, Sanofi-Pasteur, The Medicines Company, VH Squared, Viropharma. CML and AK are full time employees of Astellas Pharma Europe. All other authors – none to declare

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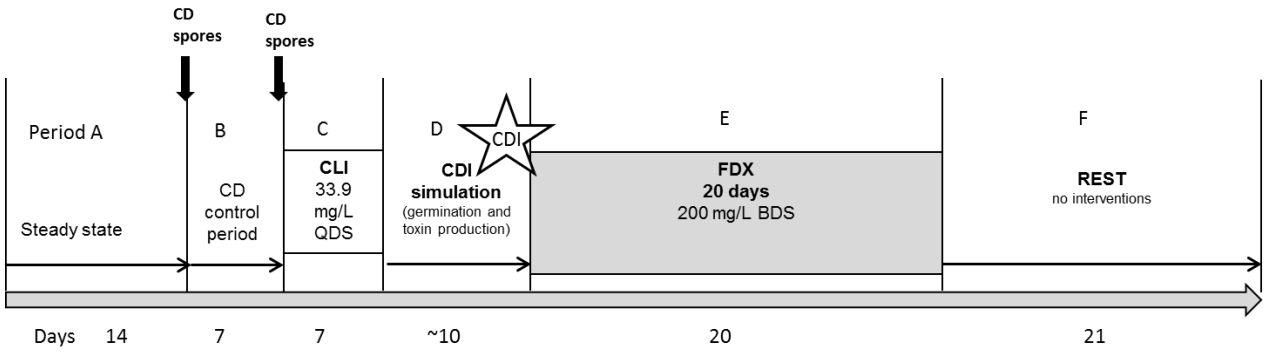
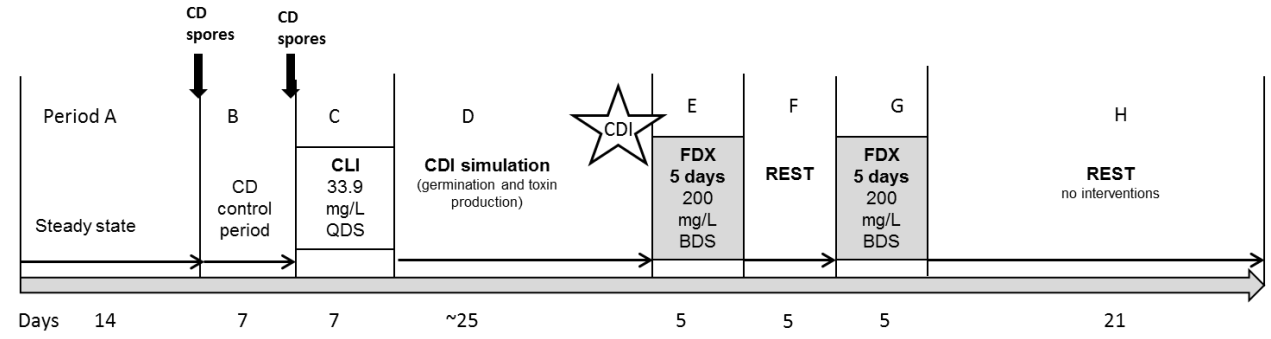
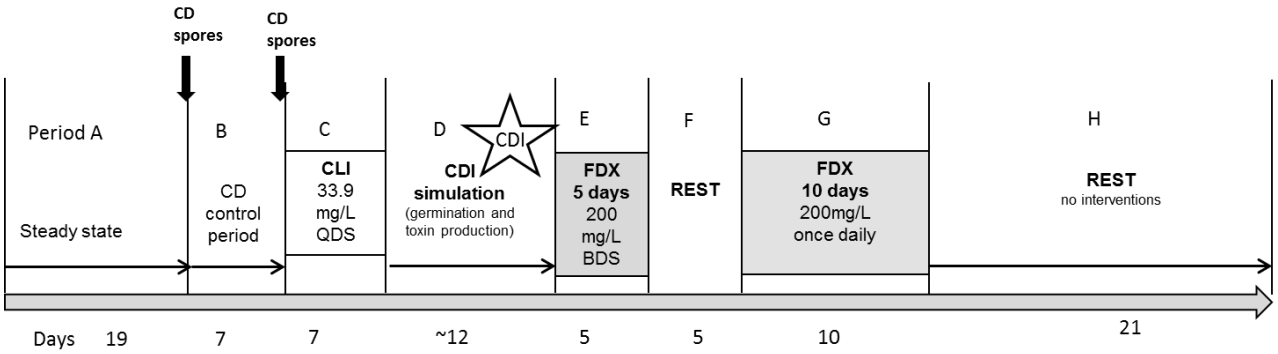
Model 1 (extended dosing)



Model 2 (pulsed dosing)



Model 3 (pulsed-tapered dosing)



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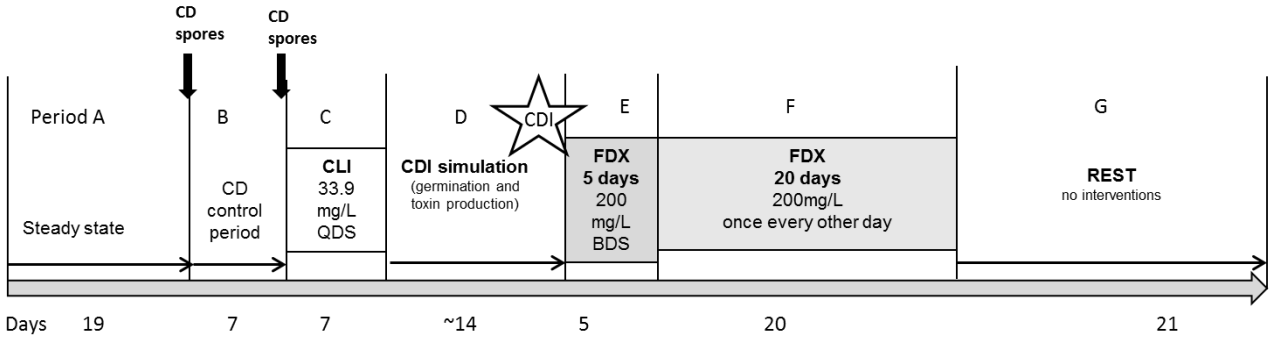
378 Model 4 (tapered-pulsed dosing)

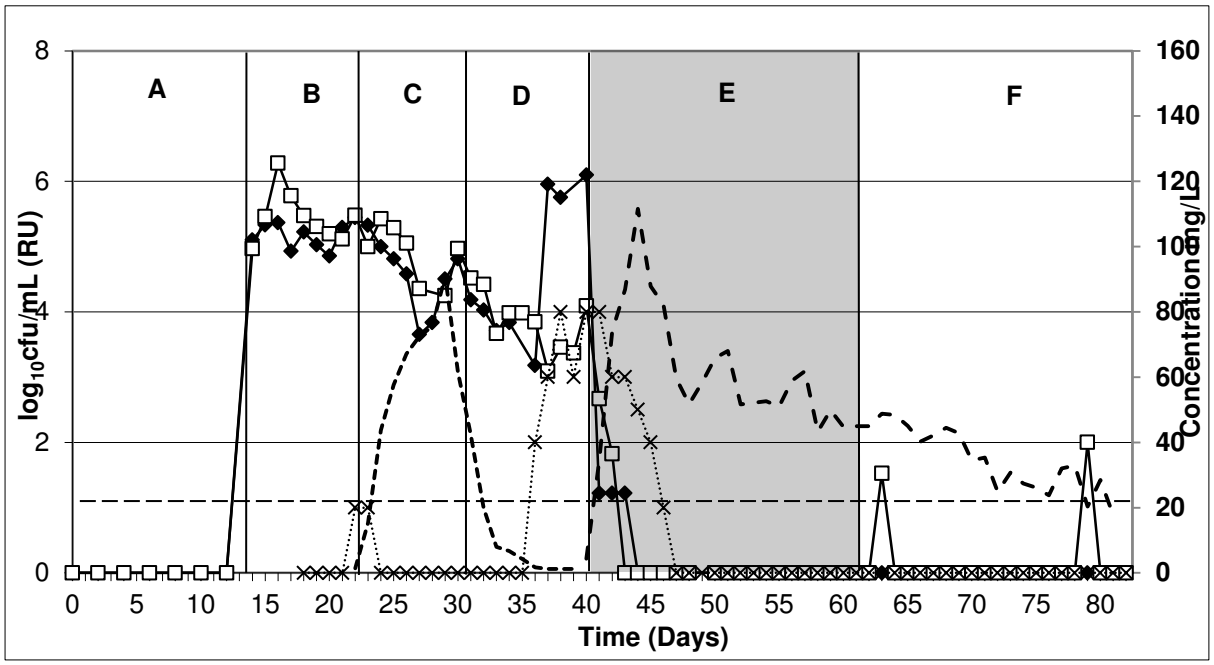


379

380

381 Figure 1 – Experimental design of the four different gut models. CLI = clindamycin instillation, FDX =
382 fidaxomicin instillation, CD spores = addition of ~10^7 cfu *C difficile* PCR ribotype 027 spores, CDI =
383 simulated *Clostridium difficile* Infection, QDS = four times daily, BDS = twice daily

384 (a)



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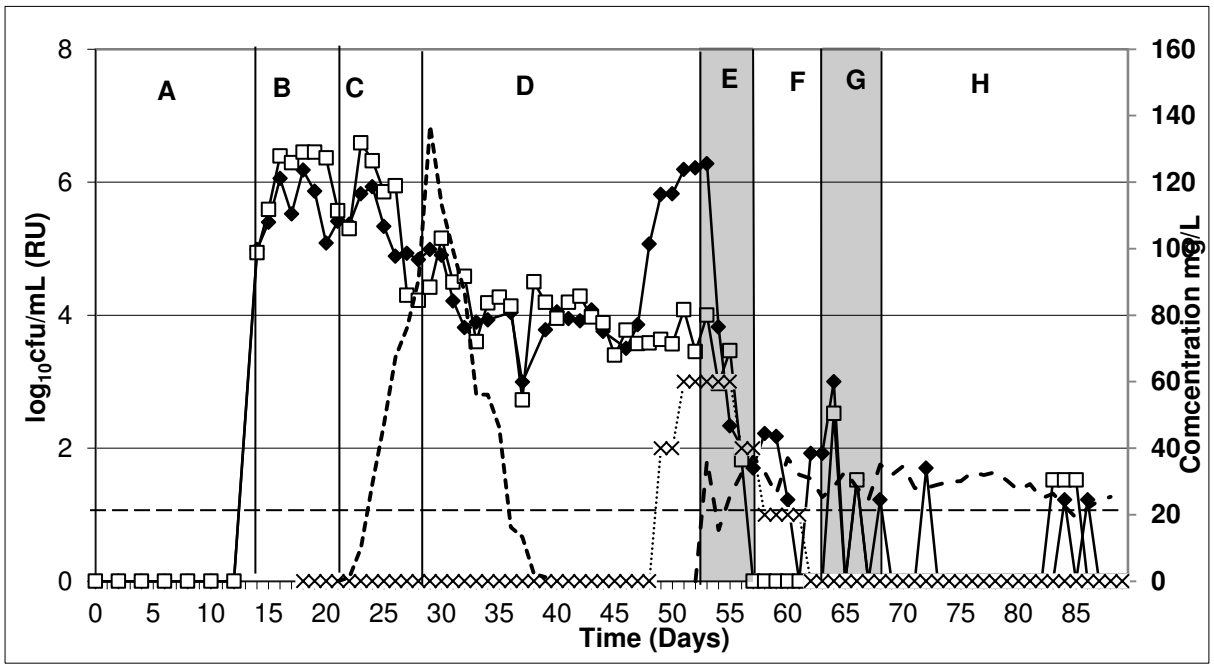
386 (b)



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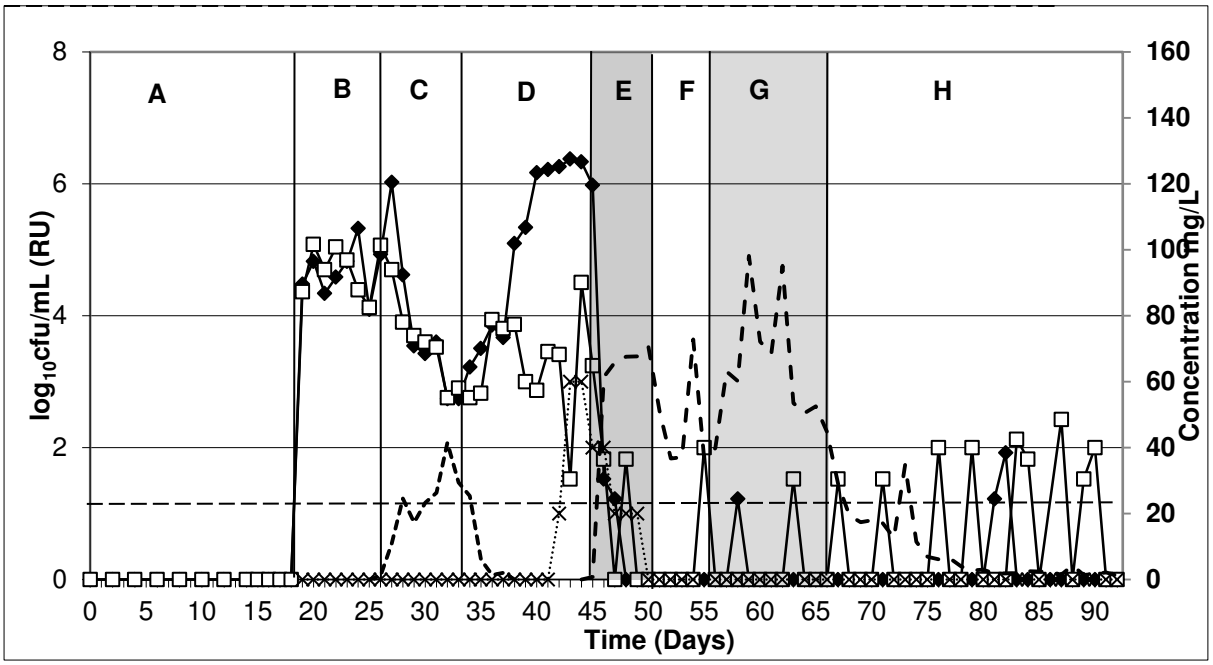
388 (c)



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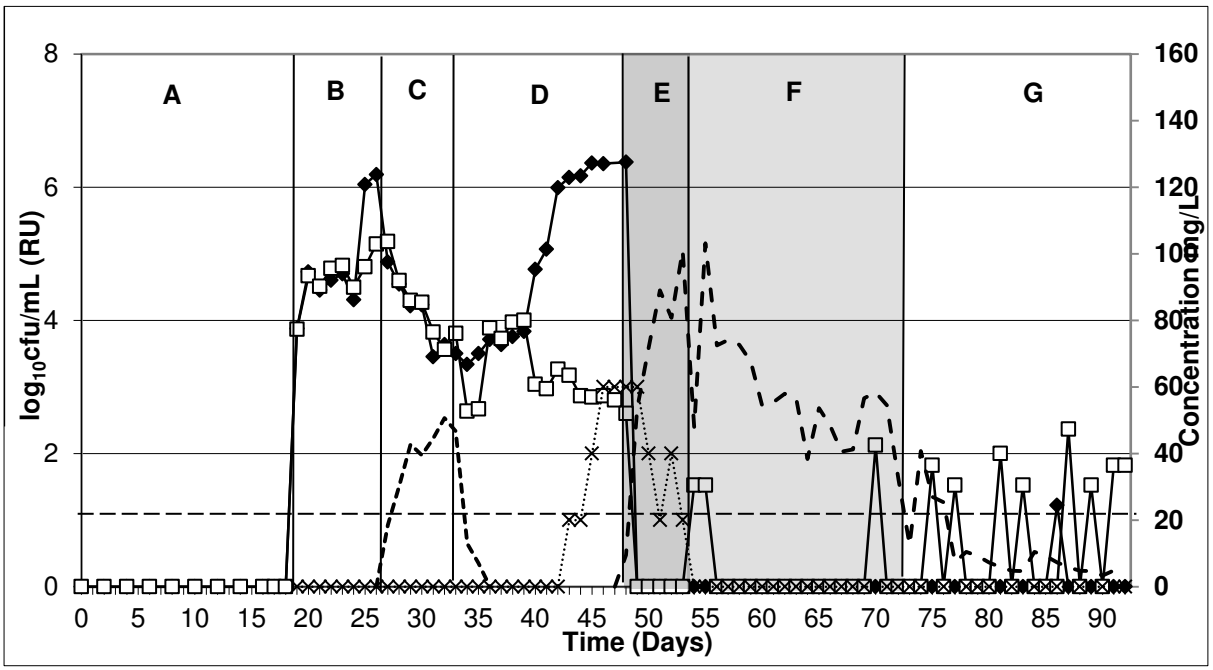
390 (d)



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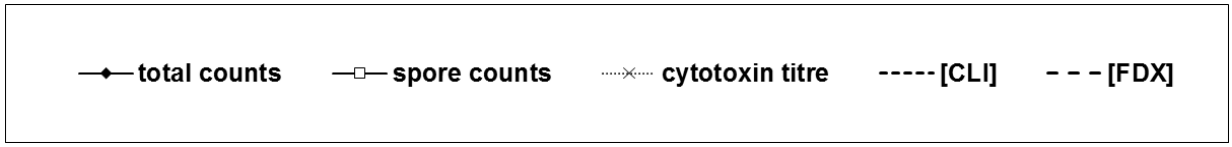
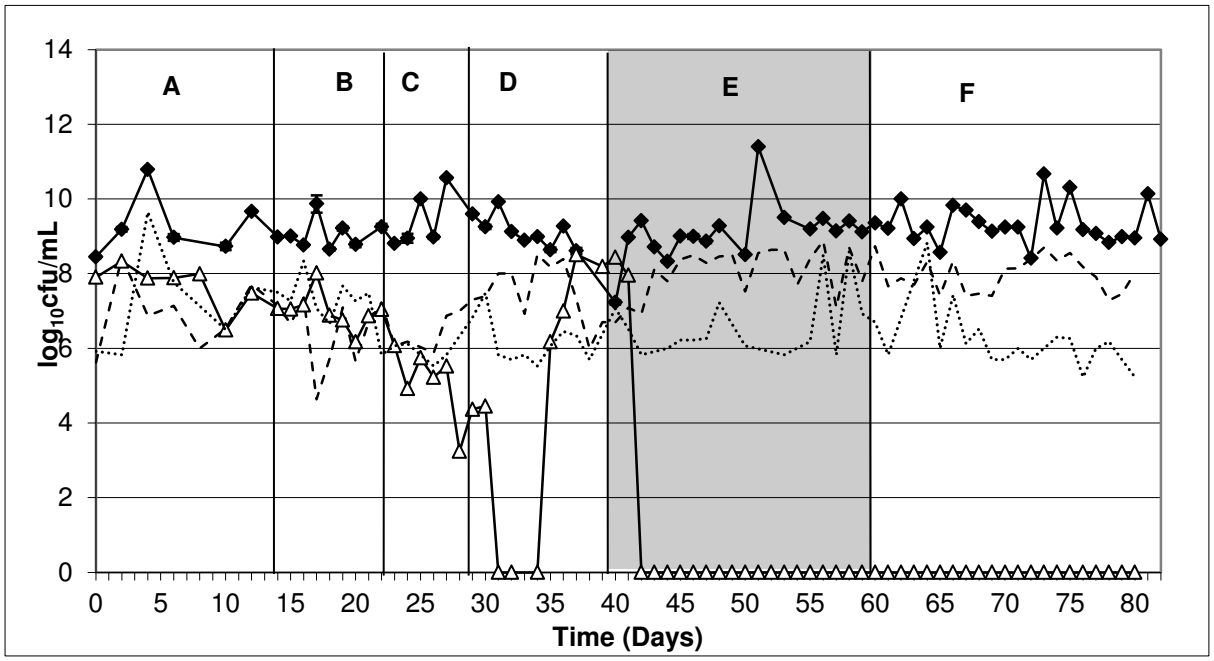


392

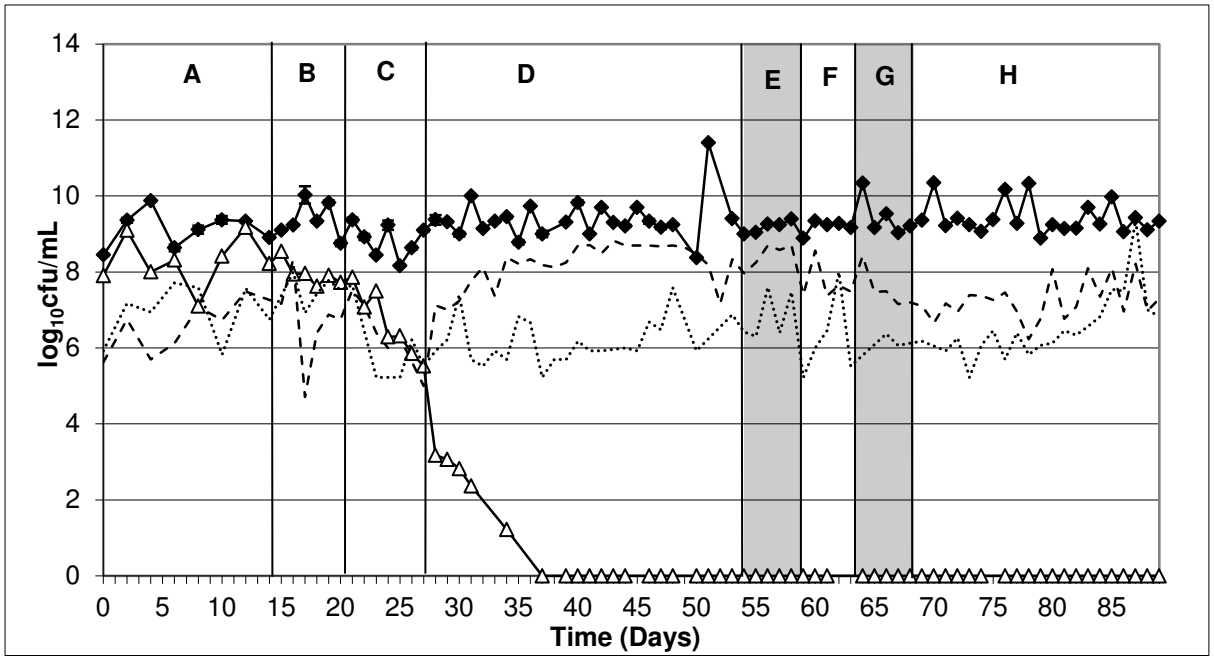
393 Figure 2. Mean *C. difficile* PCR ribotype 027 total viable counts and spore counts (log_{10} cfu/mL),
394 cytotoxin titres (relative units, RU), and antimicrobial concentration (mg/L) in vessel 3 of (a) Model 1
395 (extended dosing), (b) Model 2 (pulsed dosing), (c) Model 3 (pulsed-tapered dosing), (d) Model 4

(tapered-pulsed dosing). The broken horizontal line indicates approximate limit of detection (~1.2$\log_{10}$ cfu/mL for total counts, ~1.5 $\log_{10}$ cfu/mL for spore counts and 1 RU for toxin titre, limit of antimicrobial detection not shown). [CLI] = concentration of clindamycin, [FDX] = concentration of fidaxomicin. Periods A-H are defined in Figure 1. Treatment periods are shaded grey.

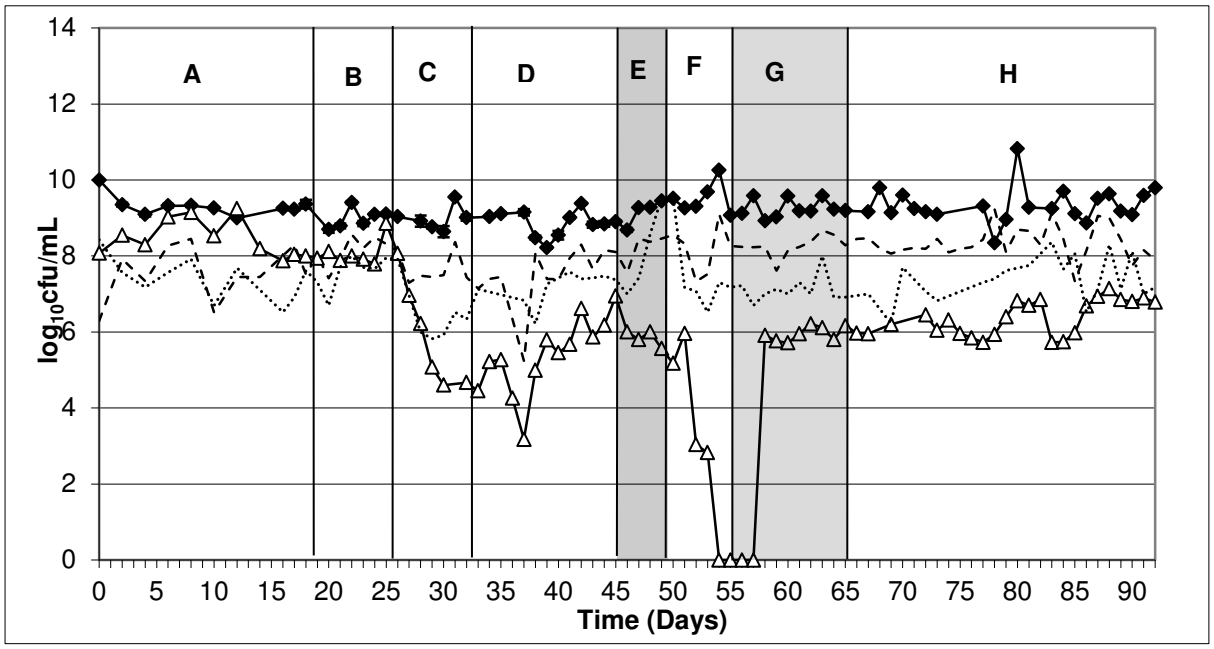
(a)



(b)



405 (c)



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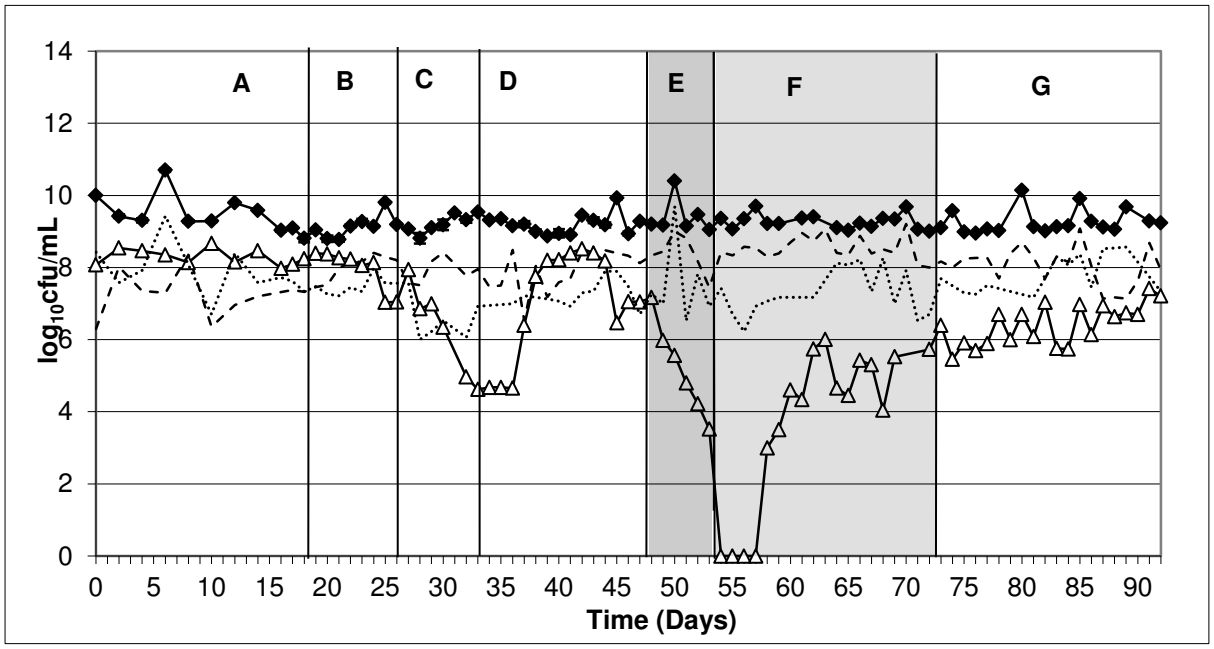
407 (d)



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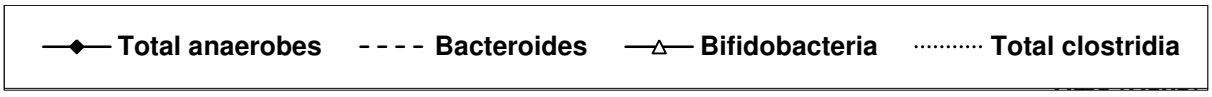
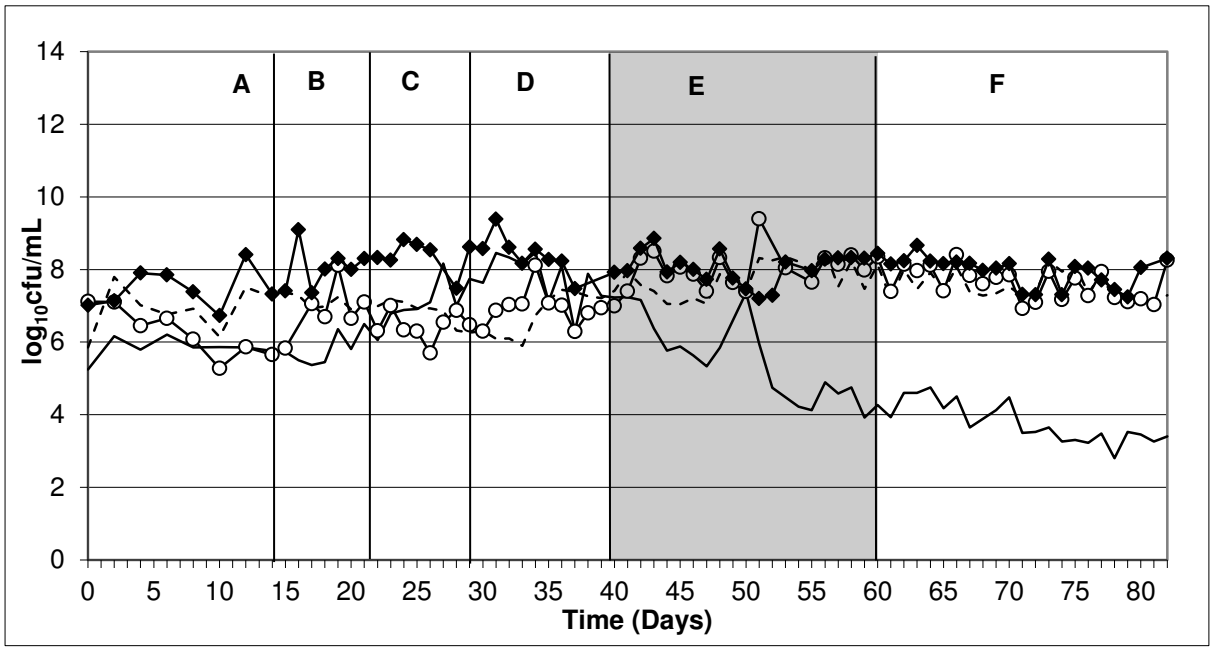


409

410 Figure 3. Mean obligate anaerobic gut microbiota populations (log_{10} cfu/mL), in vessel 3 of (a)
411 Model 1 (extended dosing), (b) Model 2 (pulsed dosing), (c) Model 3 (pulsed-tapered dosing), (d)
412 Model 4 (tapered-pulsed dosing). Periods A-H are defined in Figure 1. Treatment periods are
413 shaded grey.

414 (a)



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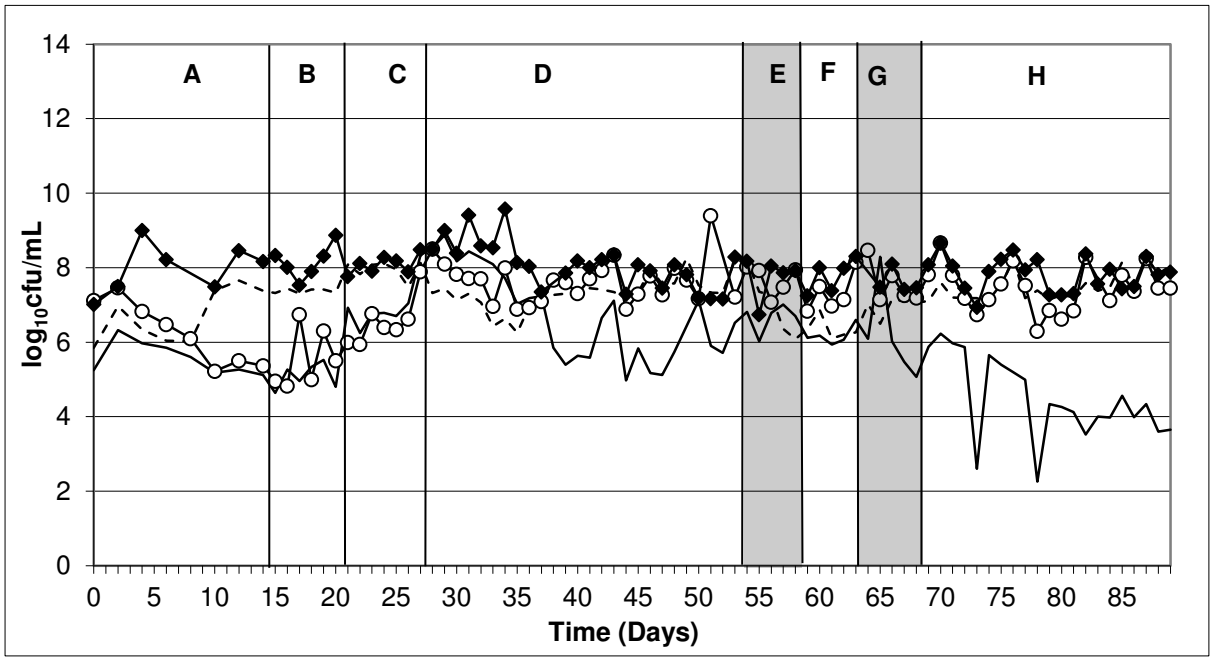
416 (b)



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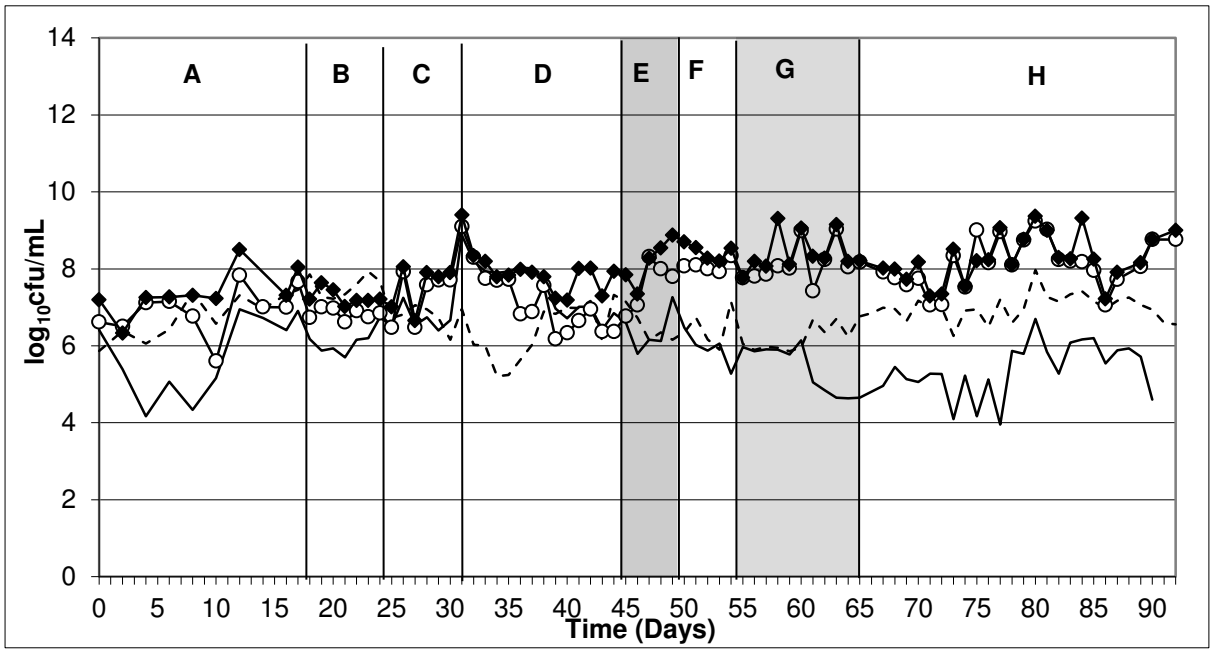
418 (c)



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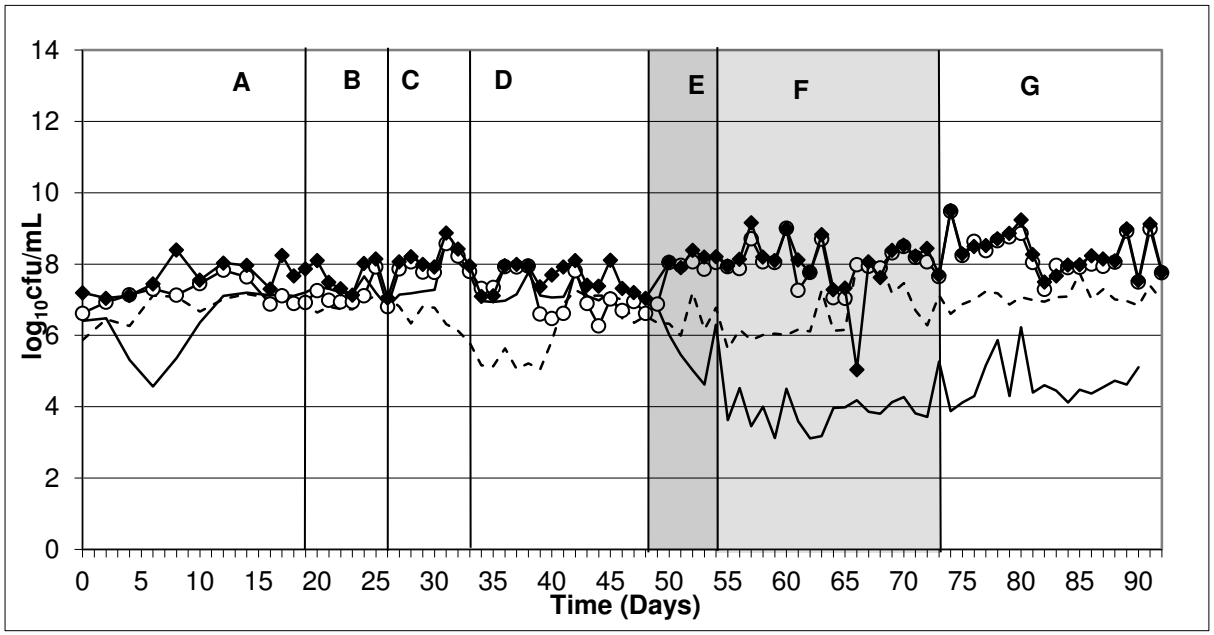
420 (d)



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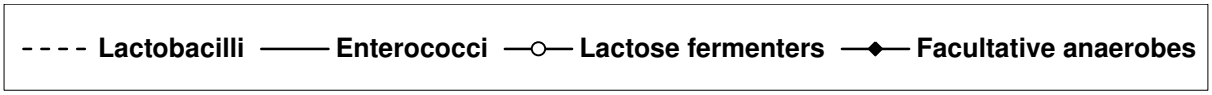

422

423 Figure 4. Mean facultative anaerobic gut microbiota populations (log_{10} cfu/mL), in vessel 3 of (a)
424 Model 1 (extended dosing), (b) Model 2 (pulsed dosing), (c) Model 3 (pulsed-tapered dosing), (d)
425 Model 4 (tapered-pulsed dosing). Periods A-H are defined in Figure 1. Treatment periods are
426 shaded grey.