Successful treatment of simulated *Clostridium difficile* infection in a human gut model by fidaxomicin first line and after vancomycin or metronidazole failure

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**Objectives:** Fidaxomicin reduces the risk of recurrent *Clostridium difficile* infection (CDI) compared with vancomycin. We investigated fidaxomicin primary or secondary treatment efficacy using a gut model.

**Methods:** Four triple-stage chemostat gut models were inoculated with faeces. After clindamycin induction of CDI, fidaxomicin (200 mg/L twice daily), vancomycin (125 mg/L four times daily) or metronidazole (9.3 mg/L three times daily) was administered for 7 days. Following failure/CDI recurrence, fidaxomicin (200 mg/L twice daily, 7 days) was instilled. *C. difficile* (CD) total viable counts (TVC), spore counts (SP), toxin titres (CYT), gut bacteria counts and antimicrobial concentrations were measured throughout.

**Results:** Fidaxomicin instillation reduced CD TVC/SP and CYT below the limit of detection (LOD) after 2 and 4 days, respectively, with no CDI recurrence. Metronidazole instillation failed to decrease CD TVC or CYT. Vancomycin instillation reduced CD TVC and CYT to LOD by day 4, but SP persisted. Recurrence occurred 13 days after vancomycin instillation; subsequent fidaxomicin instillation reduced CD TVC/SP/CYT below the LOD from day 2. CD was isolated sporadically, with no evidence of spore recrudescence or toxin production. Fidaxomicin had a minimal effect on the microflora, except for bifidobacteria. Fidaxomicin was detected for at least 21 days post-instillation, whereas other antimicrobials were undetectable beyond ~4 days.

**Conclusions:** Fidaxomicin successfully treated simulated primary and recurrent CDI. Fidaxomicin was superior to metronidazole in reducing CD TVC and SP, and superior to vancomycin in reducing SP without recurrence of vegetative cell growth. Fidaxomicin, but not vancomycin or metronidazole, persisted in the gut model for >20 days after instillation.

**Keywords:** spores, antimicrobial persistence, recurrence

**Introduction**

*Clostridium difficile* infection (CDI) continues to be a leading cause of antibiotic-associated diarrhoea¹ and a major burden on healthcare facilities worldwide.²,³ Treatment options are limited and are associated with high rates of recurrence.⁴ Fidaxomicin, a narrow-spectrum macrocyclic antimicrobial with potent bactericidal activity against *C. difficile*, was recently approved in Europe and North America for treatment of CDI. In two Phase III randomized, double-blind, clinical trials, fidaxomicin demonstrated non-inferiority to vancomycin for initial clinical cure of CDI, but superiority in reduction of recurrence and sustained clinical response (cure without recurrence during the 30 day follow-up).⁴ In this study we investigated the *in vitro* efficacy of fidaxomicin against an epidemic strain of *C. difficile* (BI/NAP1/PCR ribotype 027) in a previously validated gut model of CDI. The effects on *C. difficile* vegetative and spore forms, as well as toxin production, were quantified, alongside other components of the gut microflora. We examined the biological effects of fidaxomicin as both a primary treatment agent (after clindamycin induction of CDI) and a second-line therapy following CDI re-induction (post-fidaxomicin) or treatment failure/relapse after metronidazole or vancomycin.

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Methods

**Triple-stage chemostat gut model**

The triple-stage chemostat model used was based on that of MacFarlane et al.\(^5\) It has been validated against gut contents from sudden death victims and provides a close simulation of bacterial activities and composition in different areas of the hindgut.\(^3\) The system consists of three vessels inoculated with pooled faeces from healthy volunteers aged ≥60 years (n = 3–5), top-fed with growth medium and arranged in a weir cascade to mimic the decreasing nutrient availability of the colon from proximal to distal. All vessels are continuously stirred and regulated to reflect in vivo differences, including pH, from proximal to distal colon. Respective working volumes and pH values for vessels 1, 2 and 3 are: 280 mL, pH 5.5 (±0.2); 300 mL, pH 6.2 (±0.2); and 300 mL, pH 6.8 (±0.2). The system is sparged with oxygen-free nitrogen to maintain anaerobiosis and each vessel is fitted with a water jacket to maintain a temperature of 37°C. Growth medium (as described previously)\(^3\) is delivered to the gut model at a controlled rate using a peristaltic pump. The flow rate of 13.2 mL/h was calculated to give an overall system.

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- Model 1: clindamycin induction of CDI (Periods C and D) followed by fidaxomicin treatment (200 mg/L twice daily for 7 days; Period E).
- Model 2: clindamycin induction of CDI (Period C and D) followed by vancomycin treatment (125 mg/L four times daily for 7 days; Period E); simulation of CDI recurrence/relapse (Period F); followed by fidaxomicin treatment (200 mg/L twice daily for 7 days; Period G).
- Model 3: clindamycin induction of CDI (Period C and D) followed by metronidazole treatment (9.6 mg/L three times daily for 7 days; Period E); simulation of CDI recurrence/relapse (Period F); followed by fidaxomicin treatment (200 mg/L twice daily for 7 days; Period G).
- Model 4: clindamycin induction of CDI (Periods C and D) followed by fidaxomicin treatment (200 mg/L twice daily for 7 days; Period E); simulation of CDI recurrence/relapse (Period F) or re-induction with clindamycin if necessary (Periods G and H); followed by fidaxomicin treatment (200 mg/L twice daily for 7 days; Period I).

All models were monitored for a further 3 week period following the final intervention to investigate further recurrence.

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

The MICs of fidaxomicin for the *C. difficile* 027 210 strain used in these gut model experiments and an *Enterococcus faecalis* strain isolated from the model were determined using agar incorporation. These were found to be 0.25 and 2 mg/L for *C. difficile* and *E. faecalis*, respectively. Breakpoint agars containing four times the MIC were used to monitor reduced susceptibility, with *C. difficile* monitored on Biazier’s CCEYL containing 1 mg/L fidaxomicin in addition to the usual supplements, and enterococci monitored on kanamycin-cesulcin azide agar containing 8 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 sterilized by filtration through 0.22 μm syringe filters before being stored at −20°C. Bioassay agar (100 mL) was sterilized by autoclaving, cooled to 50°C, inoculated with 1 mL indicator organism suspension and transferred aseptically into 245 x 245 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 number 5 cork borer. Thirty microlitres of antibiotic calibrator or filter-sterilized sample from the gut model was randomly assigned to bioassay wells. 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 square zone diameters and unknown concentrations from culture supernatants determined. All assays were performed in triplicate.

For determination of clindamycin concentrations, Wilkins-Chalgren agar was inoculated with *Kocuria rhizophila* (ATCC 9341), and a calibration series ranging from 4 to 256 mg/L was used. The limit of detection of this bioassay was ~2 mg/L.

For determination of vancomycin concentrations, Mueller–Hinton agar supplemented with para-aminobenzoic acid was inoculated with *Staphylococcus aureus* (ATCC 29213). A calibration series of 8–512 mg/L was used, and the limit of detection of this bioassay was ~8 mg/L.

For determination of metronidazole concentrations, *Clostridium spores* were inoculated overnight in brain heart infusion broth (BHI) and used to inoculate Columbia Blood Agar. This was incubated anaerobically for 48 h at 37°C A calibration series of 0.25–16 mg/L was used, and the limit of detection of this bioassay was ~1 mg/L.

For determination of fidaxomicin concentrations, Wilkins-Chalgren agar was inoculated with *K. rhizophila* (ATCC 9341) and a calibration series ranging from 4 to 256 mg/L was used. The limit of detection of this bioassay was ~4 mg/L.

**Results**

Vessel 3 of the gut model is most reflective of the distal colon, and so the vessel of most clinical relevance with respect to CDI. Therefore only vessel 3 data are presented here.

**C. difficile total viable counts, spore counts and cytotoxin**

Clindamycin instillation induced *C. difficile* spore germination and toxin production 10–14 days after commencement in all four models. Toxin reached a titre of 3–4 RU (Figure 2).

In Models 1 and 4, primary fidaxomicin instillation immediately reduced vegetative cell and spore counts and toxin titre to below

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the level of detection (Figure 2). *C. difficile* was not detected for the duration of fidaxomicin instillation (Period E) and was detected only sporadically in Period F.

In Model 2, toxin titre and vegetative cell counts had reduced slightly before vancomycin instillation, but once vancomycin instillation commenced vegetative cell counts rapidly decreased to the

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**Figure 1.** Schematic of experimental design: fidaxomicin as a primary treatment agent for CDI (Model 1); fidaxomicin as a secondary treatment agent for CDI after treatment with vancomycin (Model 2) or metronidazole (Model 3); and fidaxomicin as a primary treatment with a secondary fidaxomicin treatment period (Model 4). CD, *C. difficile*; CLI, clindamycin; FDX, fidaxomicin; MET, metronidazole; VAN, vancomycin; qds, four times daily; bds, twice daily.
Figure 2. Mean *C. difficile* PCR ribotype 027 total viable counts and spore counts (log_{10} cfu/mL) and cytotoxin titres (RU) in vessel 3 of (a) Model 1, (b) Model 2, (c) Model 3 and (d) Model 4. The broken horizontal line indicates the 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). Periods A – J are defined in Figure 1. Treatment periods are shaded grey, and treatment drugs are indicated in the top left corner. FDX, fidaxomicin; VAN, vancomycin; MET, metronidazole.
limit of detection. Toxin further decreased to undetectable levels by the end of the vancomycin instillation period. Spores and total viable counts continued to be recovered at around the limit of detection for \( \approx 13 \) days, with no detectable toxin in all three vessels (Figure 2b). With no further interventions, recurrence of vegetative growth and toxin production occurred 13 days after the end of vancomycin instillation. Total viable *Clostridium difficile* counts reached \( \approx 6.5 \log_{10} \text{cfu/mL} \) with a toxin titre of 3 RU in vessel 3 (Figure 2b).

In Model 3, metronidazole instillation had little observable effect on *C. difficile* vegetative cell counts in vessel 3 (Figure 2c), and toxin titre remained at 3 RU during metronidazole instillation (Period E) and throughout the following 7 day rest period (Period F). Following recurrence in Model 2 and treatment failure in Model 3, fidaxomicin instillation resulted in an immediate reduction in *C. difficile* vegetative cell counts to below the limit of detection. Cytotoxin was no longer detectable by the end of the 7 day instillation period (Period G), and was not detected thereafter. In both models, fidaxomicin instillation prevented the recovery of any spores or vegetative cells until the final week of the experiment, when they were detected only sporadically (Figure 2).

In Model 4, *C. difficile* spores were detected at \( \approx 3 \log_{10} \text{cfu/mL} \) towards the end of Period F, but as total viable counts were equal to spore counts, this did not appear to be due to recurrence of germination and proliferation. The additional aliquot of *C. difficile* PCR ribotype 027 spores added at the start of Period G remained as spores during clindamycin instillation (Period G) until 9 days post-clindamycin instillation, when germination occurred in vessels 2 and 3. This was accompanied by toxin production, which reached a titre of 3 RU in vessel 3 (Figure 2d). Secondary fidaxomicin instillation immediately reduced *C. difficile* vegetative cell and spore counts to below the limit of detection, where they remained until the final week of the experiment (Period J), when spores were detected at \( \approx 2 \log_{10} \text{cfu/mL} \). Toxin was no longer detectable 4 days into secondary fidaxomicin instillation and thereafter.

**Gut microflora viable counts**

In all models, gut microflora populations stabilized during Period A and remained steady throughout control Period B. Clindamycin instillation caused major declines in bifidobacteria populations (\( \approx 6–8 \log_{10} \text{cfu/mL} \)) (Figure 3) and smaller declines in lactobacilli populations (\( \approx 2–4 \log_{10} \text{cfu/mL} \)) (Figure 4). Both populations recovered to steady-state levels by the end of Period D, with the exception of bifidobacteria populations in Model 4, which were detected only sporadically following clindamycin instillation. Increases were observed in populations of lactose-fermenting Enterobacteriaceae and enterococci (Figure 4). The second clindamycin instillation period in Model 4 (Period G) again caused a decline in lactobacilli populations (\( \approx 2 \log_{10} \text{cfu/mL} \)) and increases in enterococci populations (\( \approx 4 \log_{10} \text{cfu/mL} \)). Bifidobacteria populations in this model were undetectable from the start of Period G and remained below the limit of detection for the remainder of the experiment (Figure 3d).

Vancomycin instillation in Model 2 (Period E) caused a large decline in bifidobacteria (\( \approx 8 \log_{10} \text{cfu/mL} \)) and *Bacteroides* (\( \approx 8 \log_{10} \text{cfu/mL} \)) populations (Figure 3b), and smaller declines in clostridia (\( \approx 3 \log_{10} \text{cfu/mL} \), Figure 3b) and enterococci (\( \approx 2 \log_{10} \text{cfu/mL} \), Figure 4b). All populations in both vessels recovered to steady-state levels by the end of Period F.

Metronidazole instillation in Model 3 (Period E) had little effect on gut microflora populations, although there was an initial decrease (\( \approx 3 \log_{10} \text{cfu/mL} \)) and subsequent increase (\( \approx 4 \log_{10} \text{cfu/mL} \)) in enterococci populations in both vessels (Figure 4c).

Fidaxomicin instillation had only minor effects on microflora populations, except for bifidobacteria, which decreased (\( \approx 7 \log_{10} \text{cfu/mL} \)) to below limits of detection in Models 2 and 3 and did not recover throughout the remainder of the experiment. In Model 4, bifidobacteria populations did not recover prior to fidaxomicin instillation, and were detected only sporadically following initial clindamycin instillation (Period D) until secondary...
Figure 3. Mean obligate anaerobic gut microflora populations of (a) Model 1, (b) Model 2, (c) Model 3 and (d) Model 4. Periods A–J are defined in Figure 1.
clindamycin instillation reduced them to below the limit of detection for the remainder of the experiment (Period 6, Figure 3d). In Model 1, fidaxomicin instillation did not affect the bifidobacteria populations (Figure 3a). Enterococci populations also declined slightly (∼2 log10 cfu/mL) following fidaxomicin instillation (Figure 4a).

Reduced susceptibility
No C. difficile were isolated on breakpoint CCEYL agar throughout the experiment for any of the four models (data not shown). Enterococci were periodically recovered on breakpoint KAA5 agar (data not shown), but there was no substantial increase in the numbers of enterococci recovered following fidaxomicin instillation.

Antimicrobial concentrations
Clindamycin concentrations in vessel 3 of each model peaked between ∼40 and ∼80 mg/L (Figure 5), and similar levels were achieved in vessels 1 and 2 (data not shown). In all models, clindamycin was no longer detectable 4 days after cessation of instillation.

Vancomycin concentration in Model 2 peaked on day 50 at 332.2 mg/L in vessel 3 (Figure 5b) and ∼265 mg/L in vessels 1 and 2 (data not shown). No vancomycin was detectable 4 days after the cessation of instillation.

In Model 3, metronidazole was detectable in vessel 1 only, where it peaked at 8.7 mg/L (data not shown). No active metronidazole was detected in vessels 2 or 3 (Figure 5c).

Fidaxomicin concentrations peaked at ∼40–90 mg/L in vessel 3 in all models (Figure 5) and similar levels were detected in vessels 1 and 2 (data not shown). Unlike clindamycin and vancomycin, fidaxomicin persisted within the model and was still detectable at levels of 5–10 mg/L in vessel 3 of Models 1, 2, and 3 on the final day of the experiment, 21 days post-cessation of instillation (Figure 5a–c). In Model 4, fidaxomicin also persisted (whereas clindamycin did not), but to a lesser extent. Levels decreased to around 1 mg/L on day 58 in the first instillation period and day 112 in the second period. Zones of inhibition were seen periodically after this timepoint, but on many days fidaxomicin concentrations were below the limit of detection (Figure 5d).

Discussion
Fidaxomicin successfully resolved clindamycin-induced CDI in the gut model both as a primary treatment agent and as a secondary treatment agent after the failure of metronidazole or recurrence of infection following vancomycin. Response to fidaxomicin dosing was superior to that to metronidazole administration, which failed to reduce C. difficile cell counts or toxin levels. A similar lack of response to metronidazole in the gut model has previously been reported.7 Metronidazole dosing regimens were chosen to reflect the low faecal/biliary concentrations achieved in vivo.8 However, very little of the instilled metronidazole was detectable by bioassay, with no activity detected in either vessel 2 or vessel 3, a phenomenon also reported previously.7 The poor bioavailability of metronidazole, and in turn sub-optimal efficacy, may be because of antibiotic degradation by indigenous gut flora, particularly enterococci, as discussed by Freeman et al.7

In this model system, vancomycin and fidaxomicin were both effective in reducing the vegetative C. difficile cell counts and cytotoxin levels. Recurrent vegetative growth and toxin production was observed 13 days after the end of vancomycin instillation. In contrast, no signs of recurrence of CDI were observed following primary or secondary administration of fidaxomicin. Recurrent vegetative growth and toxin production has sometimes been observed in the 2 week period following vancomycin treatment in prior gut model experiments,9–11 and sometimes not.12 However, this variation may indicate that a 2 week observation period is not always sufficient time for recurrence to occur. Recurrent growth may also be due to factors specific to the gut microflora populations within the models, which may differ between different donors. Variation has been observed in the recovery of the gut microflora following vancomycin instillation, which typically has a marked deleterious effect on bifidobacteria, Bacteroides and enterococcal species within the gut model.9–13 Baines et al.9 reported full recovery of gut microflora following vancomycin instillation, whereas Freeman et al.11 observed continuing low populations of bifidobacteria and Bacteroides species.

Approximately one in five patients experience a recurrence of CDI following vancomycin treatment, suggesting again that host (gut microbiome) factors influence outcome.14 In two large Phase III clinical trials, fidaxomicin was associated with ∼50% reduction in rates of CDI recurrence compared with vancomycin. However, post hoc analyses showed that, for patients infected with C. difficile strain typeBI/NAP1/PCR ribotype027, the relative reduction in recurrence rate following fidaxomicin treatment was lower than observed in patients with a non-BI/NAP1/PCR ribotype 027 strain.4,15 We emphasize that the results in the present gut model experiments relate to a clinical C. difficile ribotype 027 strain. It has recently been suggested that sub-optimal response to antibiotic treatment of CDI due to C. difficile ribotype 027 may relate to increased gut flora disruption in some patients secondary to a more virulent pathogen. This theory has not yet been proven; however, if correct it suggests that patients with ribotype 027 may require a longer period to allow their gut flora to sufficiently recover.16

Fidaxomicin instillation seemed to prevent the recovery of C. difficile spores for a prolonged duration after instillation, a phenomenon not observed following vancomycin administration. A similar suppression of spore recovery from the gut model has been observed following instillation of ramosplatin17 and oritavancin,18 possibly because these antibiotics have bactericidal activity against outgrowth of germinating spores. Both of these antibiotics are related to lipid II-binding antimicrobial agents such as nisin, which has been shown to inhibit outgrowth of Bacillus spp. and Clostridium sporogenes.17,18 Further experiments have shown that oritavancin does not prevent early germination events, but prevents vegetative outgrowth of a spore, hence preventing recovery.19 The same has been shown for fidaxomicin.20,21 In addition, fidaxomicin (like oritavancin)19 has been shown to bind to spores, preventing their recovery even following repeated washing.22 This suggests that the lack of spore recovery from the gut model is due to fidaxomicin binding to spores within the vessels, and preventing subsequent outgrowth on agar for enumeration. As recurrence of C. difficile has been linked to persistence of spores,23 the spore effect of fidaxomicin may be an important factor in the prevention of recurrence.
The levels of fidaxomicin detected in the gut model were lower than those of vancomycin (Figure 5), despite similar amounts being instilled, and substantially lower than those observed in faeces during Phase II trials, where observed concentrations exceeded 1000 mg/kg faeces (1396 ± 1019 mg/kg).24 This may possibly be due to the ‘sticky’ nature of fidaxomicin resulting in adherence to glassware.25 However, detected fidaxomicin concentrations in the model still far exceeded the MICs for C. difficile strains reported to date (and for the isolate used in these experiments; 0.25 mg/L). Interestingly, we have observed persistence of fidaxomicin activity for a prolonged duration following cessation of instillation, whereas clindamycin and vancomycin quickly wash out of the model (metronidazole activity was not detected in vessel 3). Prolonged bioactivity may be due to sequestration of fidaxomicin into

![Graph](image)

**Figure 4.** Mean facultative anaerobic gut microflora populations of (a) Model 1, (b) Model 2, (c) Model 3 and (d) Model 4. Periods A–J are defined in Figure 1.
biofilm, with subsequent slow release. Solid biofilm visibly forms on the glass walls of the gut model vessels and is periodically shed back into the planktonic fluid. Biofilm structures may create a reservoir for fidaxomicin collection and an extended surface area for adherence, and this pool of fidaxomicin could be released back into the planktonic fluid over time. Observed spikes in fidaxomicin concentrations during this prolonged period of persistence could correspond to sloughing of biofilm structures into the planktonic fluid. We have observed high levels of fidaxomicin in biofilm removed from the vessel walls post-experiment, supporting this idea. It therefore seems probable that sequestration into the biofilm, rather than adherence to glassware, is the major factor in persistence of active antimicrobial.

The persistence of fidaxomicin observed in the gut model is consistent with Phase I human volunteer studies, in which fidaxomicin remained detectable in stool samples collected up to 5 days after a single dose. This persistence may also be important in preventing recurrence. Once vancomycin has washed out of the model, any residual spores are able to germinate. This is likely to explain the continuing detection of spores at ~2 log10 cfu/mL during Periods E and F within Model 2. Due to the chemostat nature of the system, the stable level of spores during this time is indicative of turnover, low-level germination and subsequent sporulation. However, following fidaxomicin instillation, persisting antimicrobial could continue to bind to spores, preventing any turnover and facilitating the washout of spores from the system.

Antibiotic persistence may allow recovery of gut microflora populations unaffected by fidaxomicin whilst preventing any recurrent vegetative C. difficile growth. If persistence occurs to the same extent in the gastrointestinal tract in vivo, fidaxomicin could be active against C. difficile long after dosing has stopped. Notably, in Phase III studies the reduced rate of recurrence seen with fidaxomicin in comparison with vancomycin predominantly occurred in the first 2 weeks following treatment cessation; it is plausible that this may, at least in part, relate to persistence of antimicrobial activity after therapy. Microbiota diversity studies from patients in Phase III studies have shown that recovery of the colonic microflora begins during fidaxomicin therapy. This is in contrast to vancomycin therapy, which further disrupts the anaerobic microflora (shifting the flora towards enterobacteria/lactobacilli), and does not allow its recovery until well after the end of therapy. Therefore, persistence of fidaxomicin after the end of therapy may have the dual benefit of extending the suppressive effect on CD while allowing further time for the bowel flora to recover.

Lower fidaxomicin concentrations were observed in Model 4 than the other three models during the prolonged persistence period after installation had ceased. The reasons for this are unclear, as the same dosing regimens were used. Interestingly, this model also had a higher proportion of spores recovered following cessation of fidaxomicin installation (Figure 2d), suggesting that the lower concentrations of persisting fidaxomicin may suppress spore recovery less effectively than higher concentrations, allowing increased spore recovery. Fidaxomicin installation was relatively sparing of the gut microflora population, with the exception of bifidobacteria populations, which quickly declined to below the limit of detection in three of the four models (2, 3 and 4) and did not recover, which again is likely to be linked to the observed persistence of fidaxomicin. We speculate that the composition of bifidobacteria populations established from the donor stools was different in Model 1, which may explain the apparent resistance in this model. However, bifidobacteria counts following fidaxomicin instillation did not appear to correlate with the efficacy of the drug against simulated CDI or affect whether infection recurred. Interestingly, other authors have reported that levels of bifidobacteria were not significantly affected by fidaxomicin. This may again indicate differences in bifidobacteria subgroups of individual microflora populations or a difference between the in vivo and in vitro growth of bifidobacteria. Alternatively, this discrepancy may be due to the differences in detection methods of culture (used in this study) and the molecular identification methods reported in the other studies.

There was no evidence of emergence of C. difficile exhibiting reduced susceptibility to fidaxomicin. Some enterococci were recovered with a fidaxomicin MIC ≥ 8 mg/L, although the numbers of enterococci showing reduced susceptibility did not substantially increase following antibiotic exposure despite the prolonged duration of fidaxomicin presence in the gut models. This may indicate
Figure 5. Mean concentration of antimicrobials in vessel 3 (V3) of (a) Model 1, (b) Model 2, (c) Model 3 and (d) Model 4. CLI, clindamycin; FDX, fidaxomicin; VAN, vancomycin; MET, metronidazole. Periods A–J are defined in Figure 1.
a low propensity for the selection of resistant C. difficile or enterococcal clones despite prolonged exposure to fidaxomicin.

In summary, fidaxomicin was successful in resolving simulated CDI in an in vitro gut model when used both as a primary treatment agent and as a secondary treatment agent following failure of vancomycin or metronidazole. Fidaxomicin persists within the gut model at supra-MIC levels and prevents spore recovery, whereas vancomycin does not. These observations underscore the reduced risk of recurrent CDI associated with fidaxomicin treatment seen in vivo and suggest that, at least in part, persistence of antibiotic in gut contents after antibiotic cessation and adherence of fidaxomicin to C. difficile spores may prevent recurrences that are due to germination of residual spores.

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