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The first-in-class anti-staphylococcal antibiotic AFABICIN desphosphono is not associated with *Clostridioides difficile* infection in an in vitro human gut model

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INTRODUCTION

Antibiotic use causes disruption to the healthy human gut microbiota (termed dysbiosis). Dysbiosis can diminish colonisation resistance facilitated by the microbiota and increase susceptibility to *Clostridioides difficile* infection (CDI), which is a significant cause of mortality and economic burden. Afabicin is a pathogen-specific antibiotic that is currently in development for the treatment of staphylococcal infections [1]. The active moiety, afabicin desphosphono, exerts antimicrobial effects by disrupting the enoyl-acyl carrier protein reductase, FabI, which is essential for fatty acid biosynthesis in staphylococci. Due to the highly targeted nature of afabicin, it causes minimal temporal gut microbiota changes in animal models and healthy human subjects [2]. Here, we assessed whether the microbiota-sparing property of afabicin would limit CDI in a clinically reflective in vitro human gut model.

METHODS

- The in vitro gut model consists of three fermentation vessels arranged in a weir cascade that is top fed with growth medium at a controlled rate of 0.015 h^{-1} [3]. Each vessel is anaerobic, continuously stirred, and maintained at 37°C. pH and nutrient availability is varied across vessels to reflect the proximal, medial and distal colon, respectively
- Models were inoculated with faecal slurry consisting of pooled human faeces from healthy volunteers (≥ 60 years of age, n = 5). Following a steady state period, *C. difficile* Ribotype 027 spores (10^7 colony forming units [CFU]/ml) were added on day 14 and day 21
- Four models were treated from day 21 to day 35 with dosing emulating respective predicted exposures in human faeces as follows:
  - (i) CDI-inducing positive control fluoroquinolone antibiotic levofloxacin (149.55 mg/L once daily); **Fig 1A, 1C, 2A, 2C**
  - (ii) afabicin desphosphono high dose (35.7 mg/L twice daily); **Fig 1B, 1D, 2B, 2D**
  - (iii) afabicin desphosphono low dose (17.9 mg/L twice daily) – data not shown, results similar to model (ii)
  - (iv) vehicle control (DMSO twice daily) – data not shown, results similar to model (ii)
- Samples were taken from the models for a total of 63 days
  - CFU for major facultative and obligate anaerobe populations were determined by spread plating on various selective agar plates [3]
  - *C. difficile* total viable counts spore counts were determined using differential ethanol shock followed by plating on selective agars
  - *C. difficile* toxin levels were determined using a quantitative VERO cell cytotoxicity assay [4]

KEY FINDINGS AND CONCLUSIONS

- The conventional fluoroquinolone antibiotic levofloxacin caused extensive dysbiosis in the human gut model (Fig 1A, 1C)
  - Mean reductions from steady state (day 1 to 21) during the treatment period (day 21 to 35) of >1 $\log_{10}$CFU/ml for total facultative anaerobes (-1.8 $\log_{10}$CFU/ml), lactose fermenting Enterobacteriaceae (-3.6 $\log_{10}$CFU/ml), and lactobacilli (-2.2 $\log_{10}$CFU/ml)
- Levofloxacin-induced dysbiosis resulted in simulated CDI in the human gut model
  - *C. difficile* germinated as evidenced by up to ~2 $\log_{10}$CFU increases in viable count compared to spores (Fig 2A)
  - High *C. difficile* toxin titres (Fig 2C)
- In contrast, afabicin desphosphono treatment was associated with minimal microbiota changes in the in vitro human gut model
  - No change in mean counts for major facultative (Fig 1B) or obligate anaerobe (Fig 1D) populations assessed of >1 $\log_{10}$CFU during treatment (day 21 to 35) compared to the mean steady state (day 1 to 21)
- Colonisation resistance against *C. difficile* was maintained for afabicin treated gut models resulting in no simulated CDI
  - No difference between spore and total viable *C. difficile* counts (Fig 2B)
  - No detection of *C. difficile* toxin (Fig 2D)
- **In summary, these findings support the microbiota-sparing nature of afabicin and suggest it may limit CDI risk as compared to conventional antibiotics when used clinically**

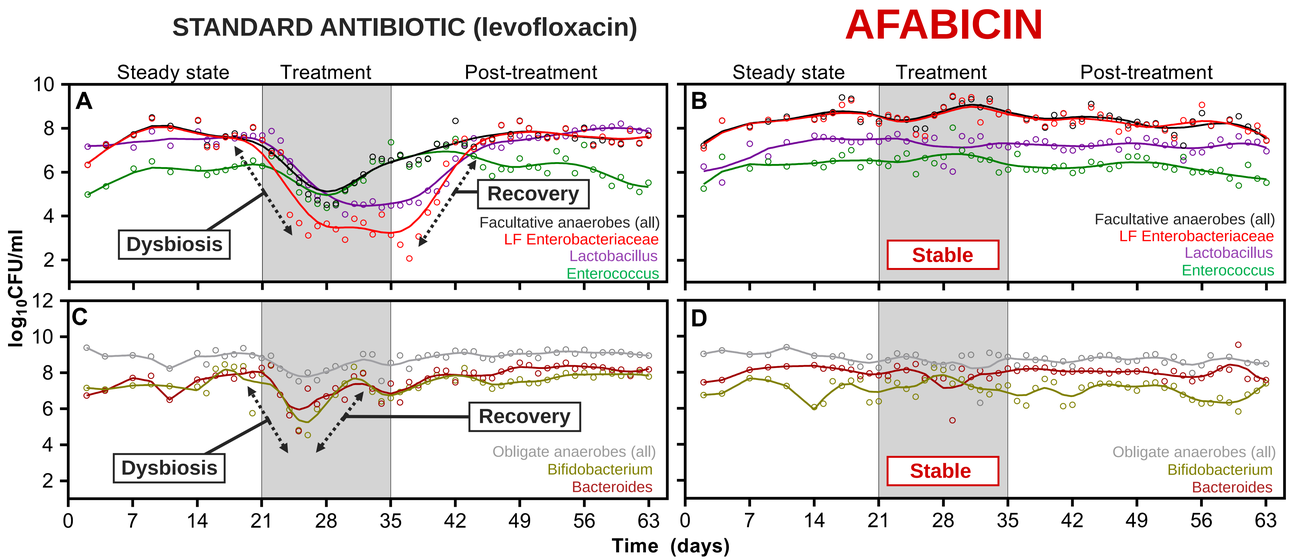

Figure 1. Mean facultative and obligate anaerobe gut populations (colony forming units, CFU) in in vitro human gut models treated with either levofloxacin (left) or afabicin desphosphono (right). Data are from vessel 3 and are the mean of triplicate measures. LF, lactose fermenting.

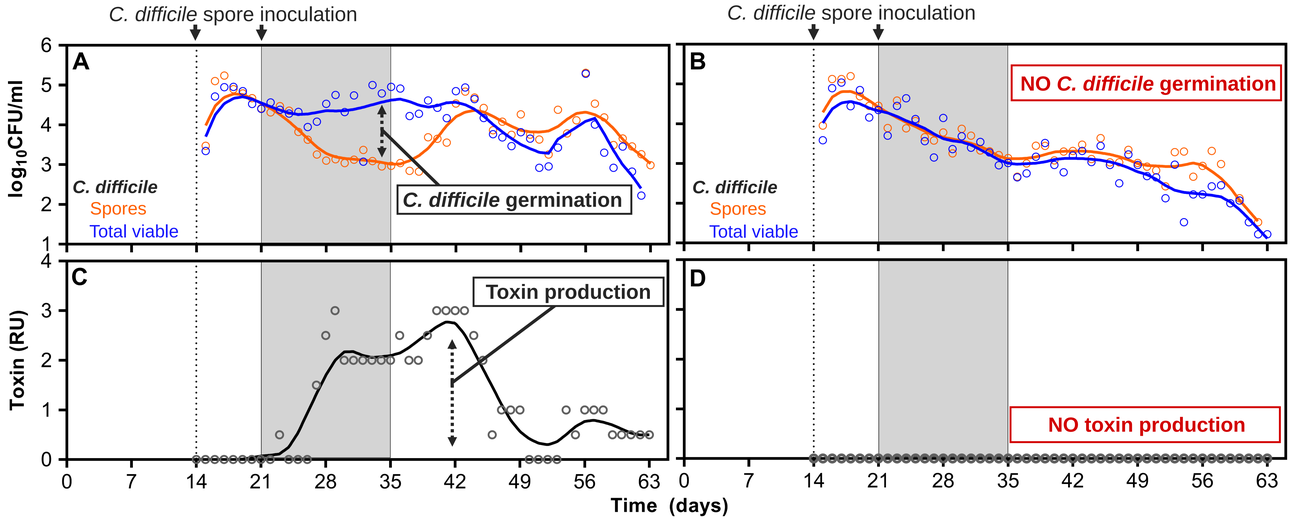

Figure 2. Mean *C. difficile* counts (colony forming units, CFU) and toxin titre (relative units, RU) in in vitro human gut models treated with either levofloxacin (left) or afabicin desphosphono (right). Data are from vessel 3. CFU are the mean of triplicate measures and RU the mean of duplicates.

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