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# OC-043 Mutation of the Ferric Uptake Regulator (FUR) Severely Impairs Toxin Production in a Human in vitro Gut Model of Clostridium Difficile Infection

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**Introduction** Regulation of iron uptake and utilisation is critical for bacterial growth and for prevention of iron toxicity. To date, little research has been carried out on iron uptake mechanisms and their regulation in *Clostridium difficile*. However, analysis of available *C. difficile* genome sequences reveals the presence of multiple iron-uptake systems and regulators underlining the importance of iron acquisition for clostridial growth. In this study, we investigated the phenotypic effects of the ferrous iron uptake system FeoB1 and the ferric uptake regulator and iron-dependent global gene regulator Fur in *C. difficile*.

**Methods** ClosTron mutagenesis was used to generate knockout mutants in a single *feoB1* and *fur* homologue in *C. difficile*630Δ*erm*, which were then inoculated into an *in vitro* human gut model to investigate relative propensity to induce *C. difficile*infection (CDI). Three parallel triple-stage chemostat gut models were primed with human faecal emulsions and spiked with *C. difficile* spores (~$10^7$ spores) from each mutant in addition to the 630Δ*erm* wild-type parental strain. Bacterial populations were allowed to equilibrate before simulated CDI was induced by instillation of clindamycin (33.9 mg/L, four times daily for 7 days). Serial samples were collected for enumeration of microflora populations, *C. difficile* vegetative cells, spores and measurement of cytotoxin titres.

**Results** Cytotoxicity assays revealed that the *fur* mutant strain produced considerably lower toxin levels (~1000 fold lower) than the *feoB1* and wild type strain. Following clindamycin exposure, all three *C. difficile* strains germinated and exhibited sustained vegetative proliferation (~5.5/6 $\log_{10}$ cfu/mL). The *feoB1* mutant strain germinated slightly earlier than the other strains, which may have been influenced by the slightly lower clindamycin levels in the *feoB1* model. Indeed, compared to wild type, higher minimum inhibitory concentrations were observed for both mutant strains, indicating reduced susceptibility to clindamycin. In all three models, the introduction of clindamycin caused a decline in Bifidobacteria (3.5 $\log_{10}$ cfu/mL), Clostridia (~3 $\log_{10}$ cfu/mL) and Lactobacilli (~2 $\log_{10}$ cfu/mL) with increases in Enterococci and Enterobacteriaceae (2–4 $\log_{10}$ cfu/mL). However, no specific microflora changes correlated with the strain of *C. difficile* used in each of the models.

**Conclusion** These findings reveal the important role of the Fur system in regulating the expression of *C. difficile* toxins. Modulation of iron homeostasis may represent a potential novel therapeutic or preventative strategy against CDI.

**Disclosure of Interest** None Declared