First line treatment in the UK for Clostridioides difficile infection can increase biofilm biomass in RT012 strains

First line treatment in the UK for *Clostridioides difficile* infection can increase biofilm biomass in RT012 strains

Megan G. Taggart[1], James S. G. Dooley[1], Nigel G. Ternan[1]
[1]*Nutrition Innovation Centre for Food & Health (NICHE), Ulster University, Coleraine, BT52 1SA*

Ulster University
NICHE
Nutrition Innovation Centre for Food and Health
ulster.ac.uk/niche

Introduction

C. difficile causes the most common healthcare-associated bacterial infection in the USA (CDC, 2019), leading to significant costs for healthcare systems (Balsells *et al.,* 2019). Current treatment options include antibiotics such as **vancomycin, metronidazole or fidaxomicin**.

Microbes form biofilms when an increase in cell density triggers cells to produce a hydrated extracellular matrix of proteins, polysaccharides and extracellular DNA which provides protection to the cells within.

Biofilms express increased resilience to antibiotics compared to their planktonic counterparts with antibiotic stress being a known inducer of biofilm formation (Olsen, 2015; Vuotto *et al.,* 2016). Specifically, biofilm formation in *C. difficile* and spore presence within biofilms have been linked to CDI persistence and recurrence (Normington *et al.,* 2021). Therefore, it is important to consider how the effects of first line antibiotic treatment on *C. difficile* biofilm could cause implications for treatment of CDI.

Aims

We aimed to determine:

- The efficacy of antibiotic treatment on *C. difficile* clinical isolates representing the most prevalent ribotypes in Northern Ireland, namely ribotypes 078, 005, 014, 015 and 002.
- The effects of antibiotic treatments on *C. difficile* RT012 strain biofilms assessed by crystal violet straining of biofilm biomass.
- Determine the most effective antibiotic against in-vitro *C. difficile* biofilms.

MIC and MBC of first line antibiotic treatments for *C. difficile*

Minimum inhibitory and biocidal concentrations were determined by broth microdilution method with growth measured by optical density at 600 nm.

MIC – defined as the minimum concentration at which there is a significant decrease in growth measured by optical density at 600 nm

Table 1. MIC and MBC of vancomycin, metronidazole and fidaxomicin required for representative strains of most prevalent ribotypes in NI

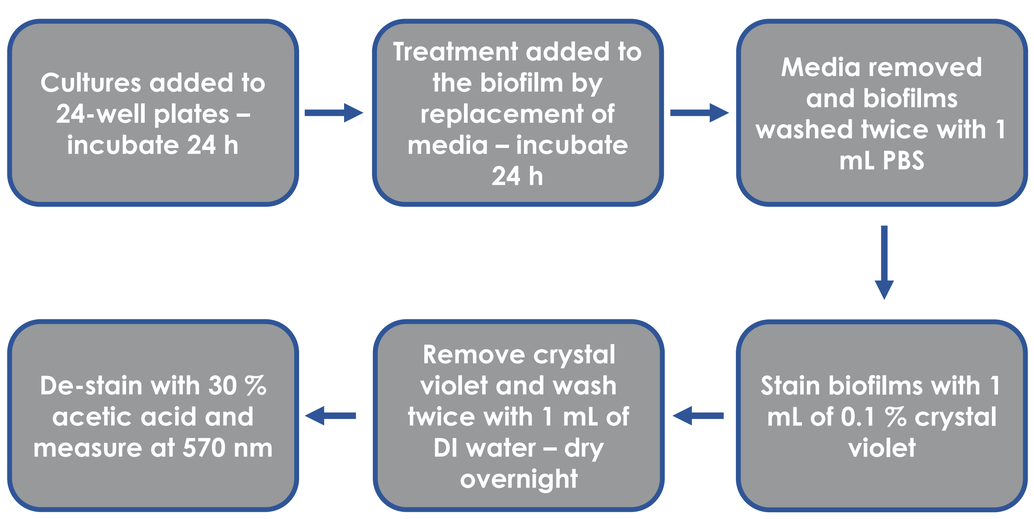
Ribotype (RT)	Vancomycin (µg/mL)		Metronidazole (µg/mL)		Fidaxomicin (µg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC
RT002	2	2	0.5	2	0.005	0.01
RT005	2	2	0.25	2	0.02	0.02
RT014	0.5	1	0.125	2	0.04	0.04
RT015	0.25	1	1	2	0.01	0.01
RT078	1	1	0.25	0.5	0.01	0.02
RT027	0.031	4	0.0625	2	0.16	0.16

Table 2. MIC and MBC of vancomycin, metronidazole and fidaxomicin required for ribotype 012 strain 630 and derived mutants

Strain (RT012)	Vancomycin (µg/mL)		Metronidazole (µg/mL)		Fidaxomicin (µg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC
630	0.0625	2	1	1	0.01	0.01
630Δ*erm*	0.125	2	0.125	2	0.005	0.02
630Δ*erm:dnaK*	1	1	0.125	0.25	0.0025	0.0025

Effects of first line treatments on *C. difficile* biofilm

Methods



Results

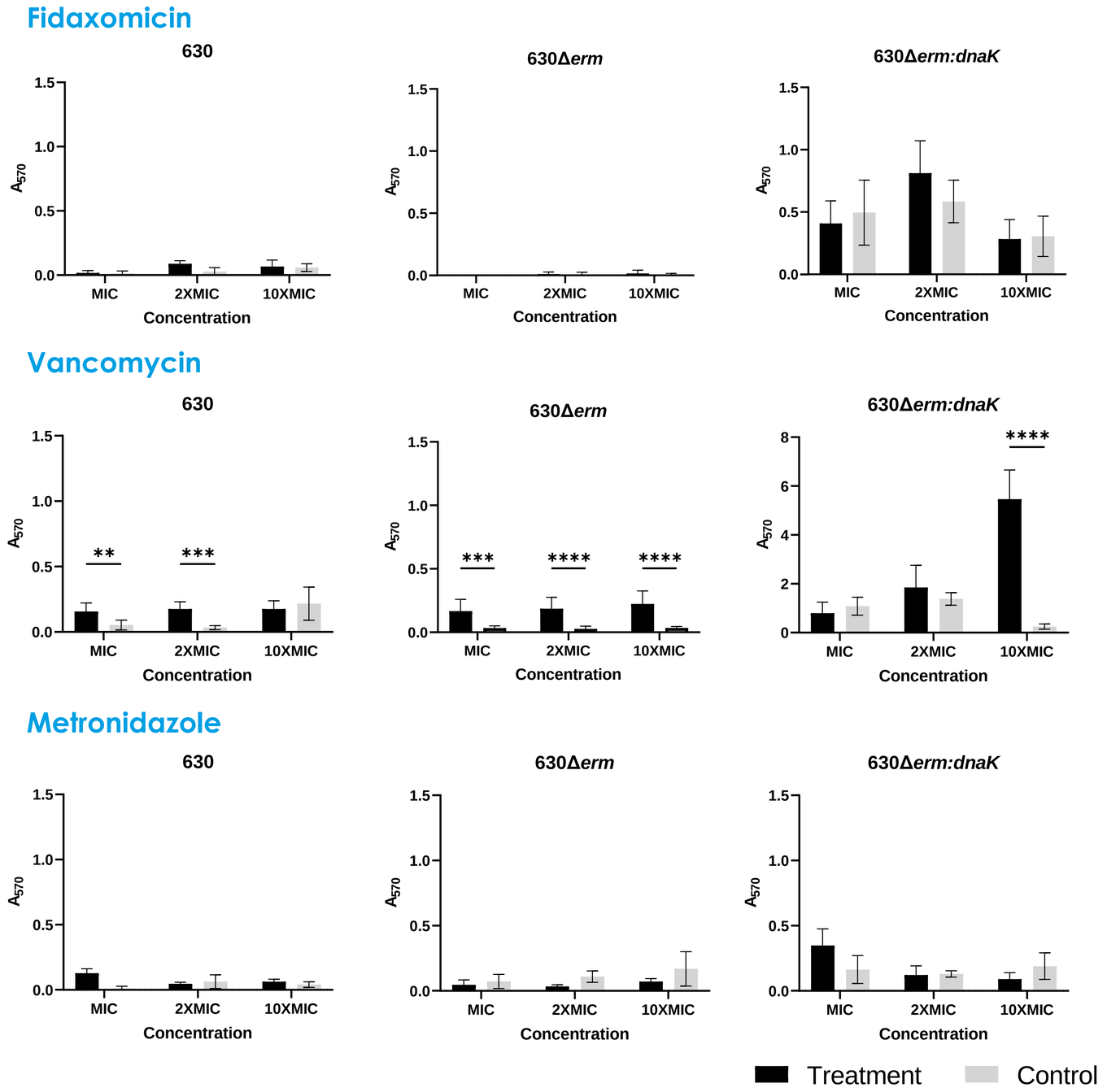


Figure 1. Biofilm biomass after treatment of 24 h biofilms with antibiotics or vehicle control (No antibiotic treatment) for a further 24 h. n = 6, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$

Conclusions

- Fidaxomicin was effective against at a much lower concentration than either vancomycin or metronidazole.
- Vancomycin being first line treatment for CDI in the UK increased *C. difficile* biofilm biomass at concentrations of MIC and above.
- Fidaxomicin had no significant effect on biofilm biomass of RT012 strains.
- Metronidazole treatment at MIC, 2X MIC and 10X MIC also had no significant effect on biofilm biomass of RT012 lineage strains.

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References. Balsells *et al.,* 2019. Global burden of *Clostridium difficile* infections: A systematic review and meta-analysis. J Glob Health. **Brown *et al.,* 2013.** Tetrazolium reduction allows assessment of biofilm formation by *Campylobacter jejuni* in a food matrix model. *Journal of applied microbiology*. **CDC, 2019.** Antibiotic resistance threats in the United States. US Dep Heal Hum Serv CDC. **Olsen, 2015.** Biofilm-specific antibiotic tolerance and resistance. European Journal of Clinical Microbiology and Infectious Diseases. **Vuotto *et al.,*** 2016. Subinhibitory concentrations of metronidazole increase biofilm formation in *Clostridium difficile* strains. Pathogens and disease. **Normington *et al.*, 2021.** Biofilms harbour *Clostridioides difficile*, serving as a reservoir for recurrent infection. npj Biofilms and Microbiomes.

@NICHE_ULSTER niche_ulster M.Taggart1@ulster.ac.uk NICHE Ulster / UlsterUniversityNICHE