Healthcare Associated Infection Research Group
FACULTY OF MEDICINE AND HEALTH

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Inhibition of bioactivity of anti-*Clostridium difficile* agents

Potentially gut microbiota mediated?

Caroline Chilton

c.h.chilton@leeds.ac.uk

This study was supported by Merck and Co., Inc., Kenilworth, NJ

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Clostridium difficile Infection (CDI)

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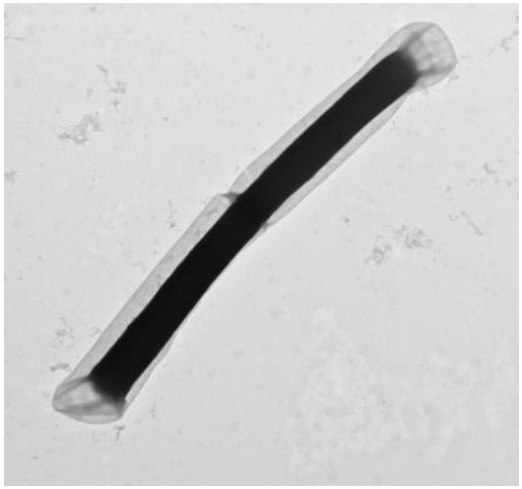
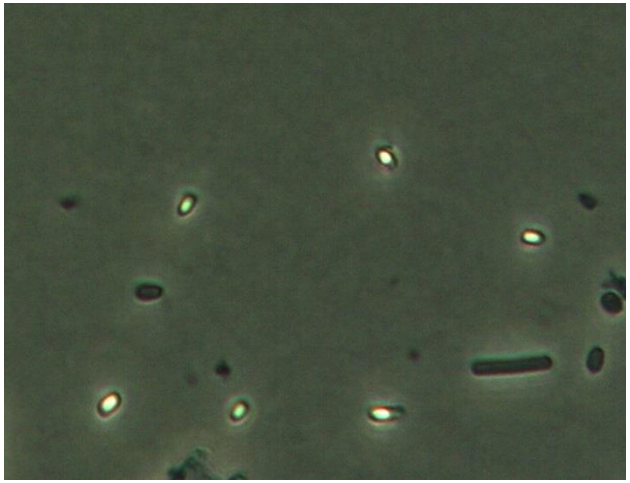
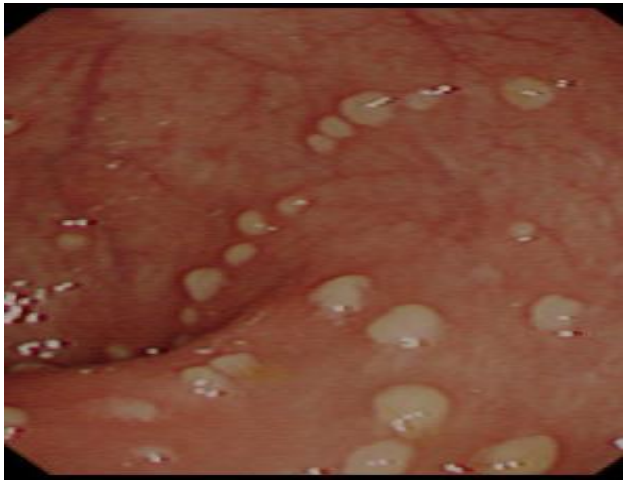
- Anaerobic, spore forming, Gram positive bacillus
- Toxin mediated disease
- Major cause of antibiotic-associated diarrhoea
- Treatment is generally antibiotics
- *Treatment failure / recurrence ~20-30% of cases*







Site of Disease

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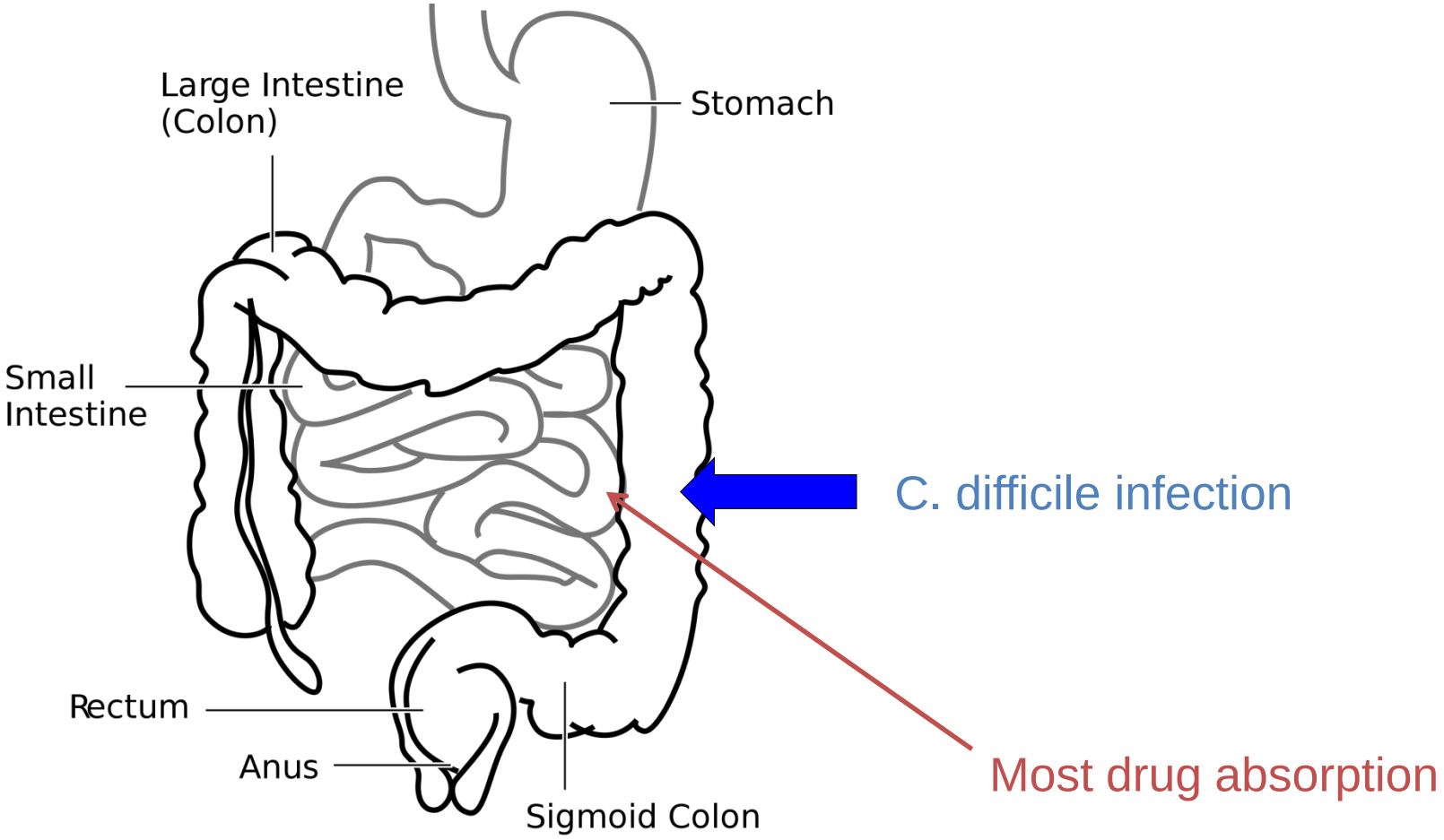

Treatment Agents

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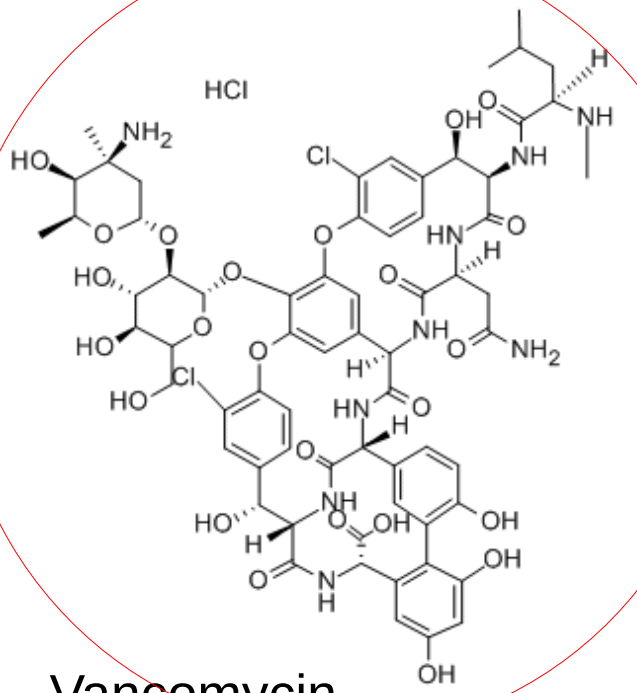
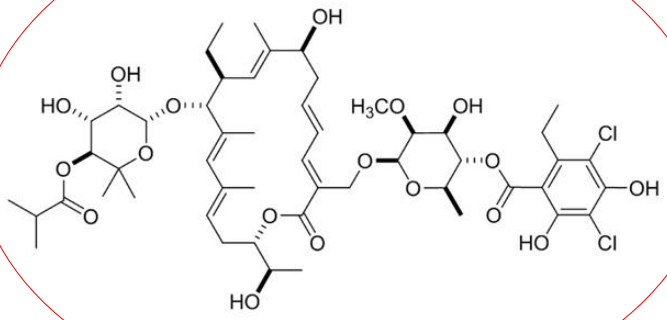
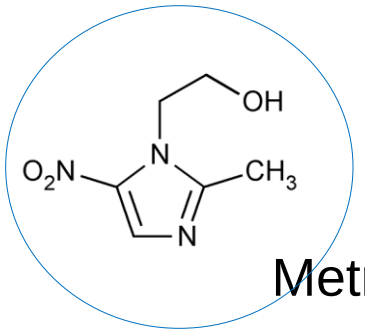


Vancomycin

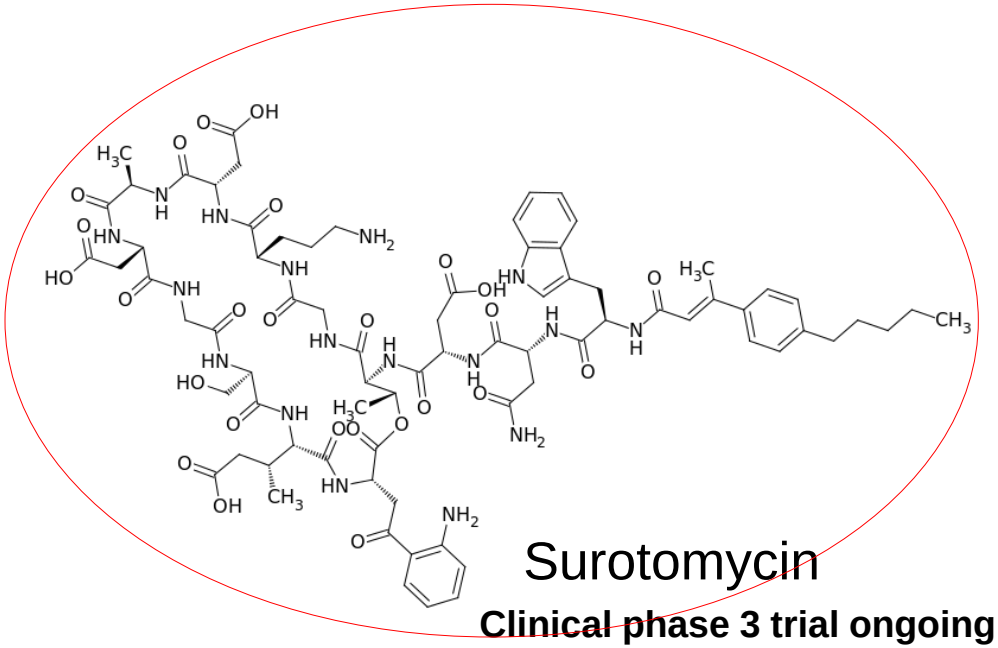


Fidaxomicin

Courtesy of Optimer Pharmaceuticals



Metronidazole



Surotomycin

Clinical phase 3 trial ongoing

MIC against *C. difficile*

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		SURO	MET	VANC	FDX
All 2013 strains	Range	0.06-2	0.125-4	0.5-4	0.015-0.25
n=100	**GeoMEAN**	**0.21**	**0.27**	**0.75**	**0.06**
2013 Ribotype 078	Range	0.25 - 2	0.125 - 0.5	0.5 - 1	0.03 - 0.125
n=10	**GeoMEAN**	**0.57**	**0.22**	**0.62**	**0.07**
2013 Ribotype 027	Range	0.125-0.5	1-4	0.5-1	0.015-0.125
n=10	**GeoMEAN**	**0.23**	**2.0**	**0.66**	**0.05**
2013 Ribotype 001	Range	0.25-0.5	0.125-1	0.5-4	0.015-0.125
n=10	**GeoMEAN**	**0.27**	**0.30**	**1.1**	**0.03**
2013 Ribotype 026	Range	0.125-1	0.125-0.25	0.5-1	0.03-0.125
n=10	**GeoMEAN**	**0.29**	**0.23**	**0.76**	**0.06**
All historical strains	Range	0.06-1	0.125-8	0.5-4	0.008-0.25
n=95	**GeoMEAN**	**0.62**	**1.3**	**0.81**	**0.04**
Historical MET sensitive	Range	0.06-1	0.25-4	0.5-4	0.008-0.125
n=30	**GeoMEAN**	**0.60**	**1.3**	**0.91**	**0.04**
Historical MET Resistant	Range	0.25-1	1-8	0.5-4	0.015-0.25
n=33	**GeoMEAN**	**0.58**	**4.6**	**0.794**	**0.04**

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	SURO	MET	VANC	FDX
Typical Faecal Levels	~250 mg/L[1]	~9 mg/L[2]	~500 mg/L[3]	~1000 mg/L[4]
Recent isolates	0.21 mg/L	0.27 mg/L	0.75 mg/L	0.06 mg/L
Historical isolates	0.62 mg/L	1.3 mg/L	0.81 mg/L	0.04 mg/L
Highest MIC	2 mg/L	8 mg/L	4mg/L	0.25mg/L

[1] Cubist Pharmaceuticals – data on file

[2] Bolton *et al,* Gut, 1986

[3] Keighley et al, BMJ, 1978

[4] Sears *et al*, Clin Infect Dis, 2012

However…

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- Faecal concentrations can vary between different individuals

Table 11: Phase 3 Fidaxomicin Plasma and Faecal Concentrations by Formulation

Sample		Formulation 1 (200 mg film coated tablets)		Formulation 2 (200 mg uncoated tablets)	
		Fidaxomicin	OP-1118	Fidaxomicin	OP-1118
Plasma (Day 1, 3-5 hours), ng/mL	N	107	106	57	57
	Mean (SD)	21.78 (27.22)	39.51 (46.71)	24.66 (25.21)	49.68 (58.87)
	Range	[0.4, 185.0]	[0.3, 321.0]	[1.1, 102.0]	[1.9, 363.0]
Plasma (Day 10, 3-5 hours), ng/mL	N	38	38	21	21
	Mean (SD)	25.28 (33.25)	68.99 (83.10)	28.40 (27.16)	72.68 (77.36)
	Range	[2.4, 191.0]	[3.0, 370.0]	[1.9, 99.70]	[4.7, 264.0]
Faecal (Day 10, 0-24 hours), ng/mL	N	65	63	37	36
	Mean (SD)	1344.55 (853.36)	711.59 (473.42)	1015.16 (500.70)	980.13 (861.55)
	Range	[31.7, 4640.0]	[63.4, 3140.0]	[102, 1960.0]	[93.7, 4170.0]

(http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002087/WC500119707.pdf)

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Faecal matter can affect activity of antimicrobials

How does faeces affect anti – CDI agents?

Methods

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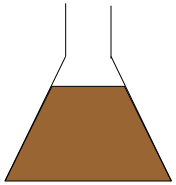
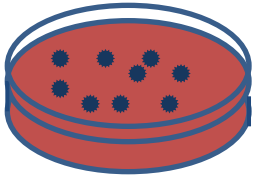
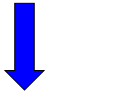
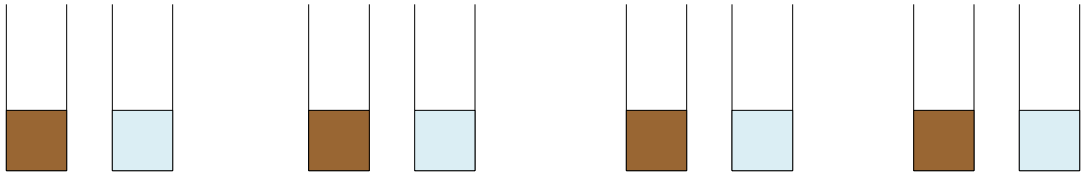


Faecal slurry 20%(w/v)



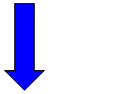
Bacterial enumeration





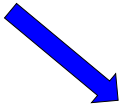
Suro (128mg/L) Met (128mg/L) Vanc (128mg/L) FDX (8mg/L)

Anaerobic
37°C
1 hour



CENTRIFUGE

Bioassay



MIC determination

Broth Microdilution

Bioassay results

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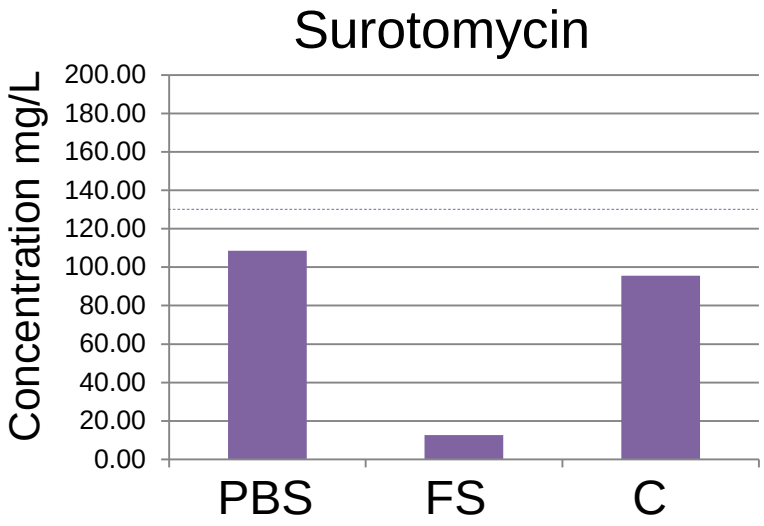

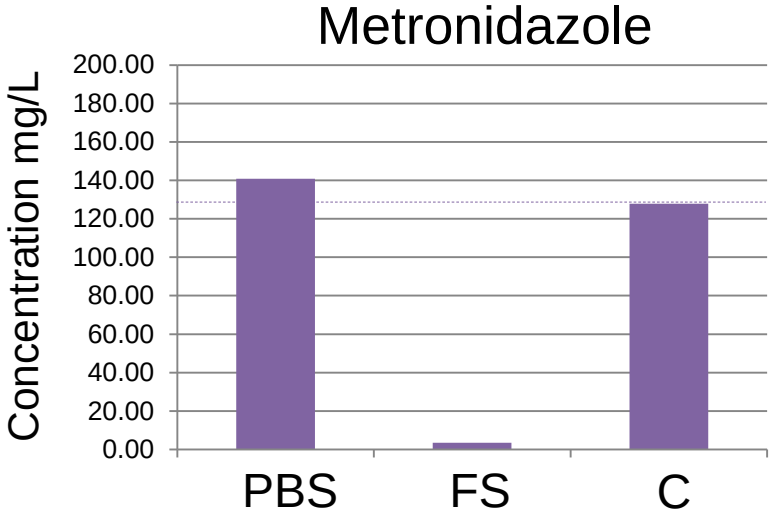

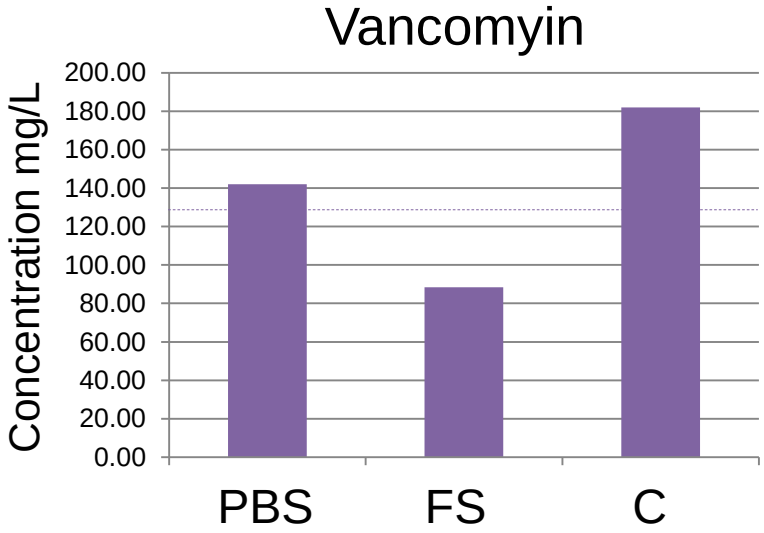

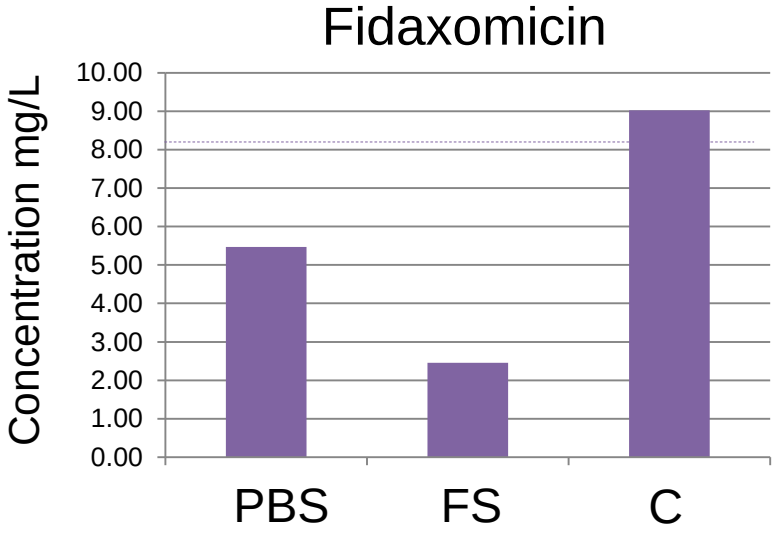

Broth Microdilution MICs

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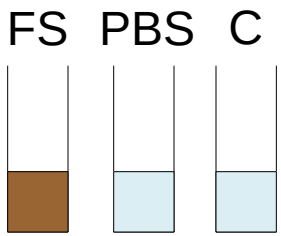

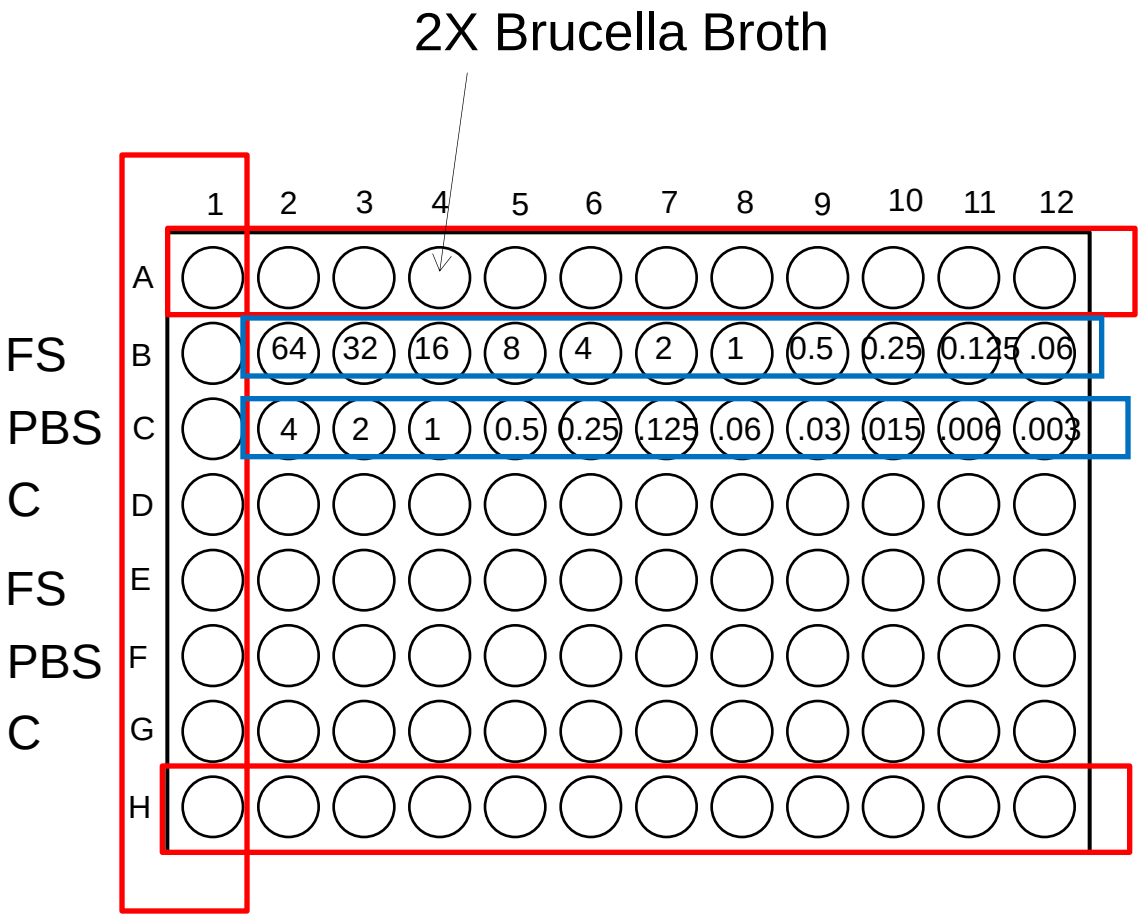

Broth Microdilution MICs

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Vancomycin Metronidazole Fidaxomicin Surotomycin

	VAN	VAN	VAN	VAN	MET	MET	MET	MET	FDX	FDX	FDX	FDX	SURO	SURO	SURO	SURO
	AI	PBS	FS	C	AI	PBS	FS	C	AI	PBS	FS	C	AI	PBS	FS	C
n=	30	27	27	26	30	27	27	27	30	28	28	27	30	28	28	27
GeoM	0.87	0.54	0.84	0.53	0.33	0.35	38.3	0.34	0.05	0.13	1.5	0.05	0.16	0.30	15.6	0.31

Broth Microdilution MICs

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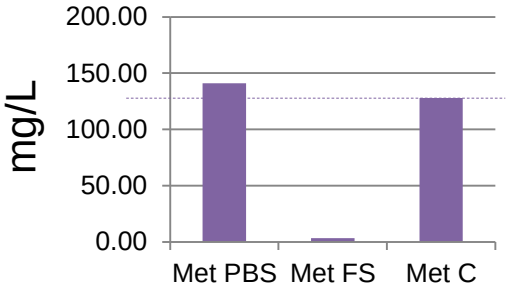
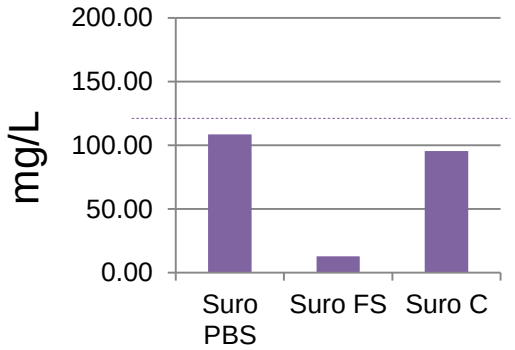
Vancomycin Metronidazole Fidaxomicin Surotomycin

	VAN	VAN	VAN	VAN	MET	MET	MET	MET	FDX	FDX	FDX	FDX	SURO	SURO	SURO	SURO
	AI	PBS	FS	C	AI	PBS	FS	C	AI	PBS	FS	C	AI	PBS	FS	C
n=	30	27	27	26	30	27	27	27	30	28	28	27	30	28	28	27
GeoM	0.87	0.54	0.84	0.53	0.33	0.35	38.3	0.34	0.05	0.13	1.5	0.05	0.16	0.30	15.6	0.31

Fold change 1.6 112 30 50





Inverse relationship – lower detectable activity = higher apparent MIC

Broth Microdilution MICs

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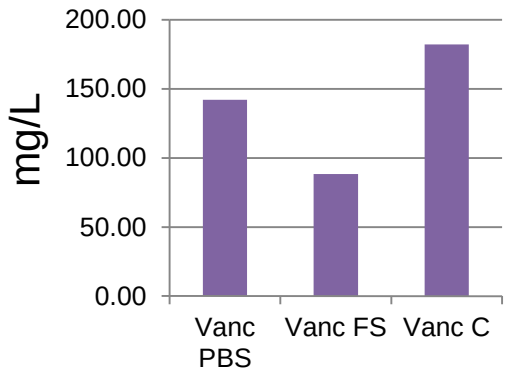
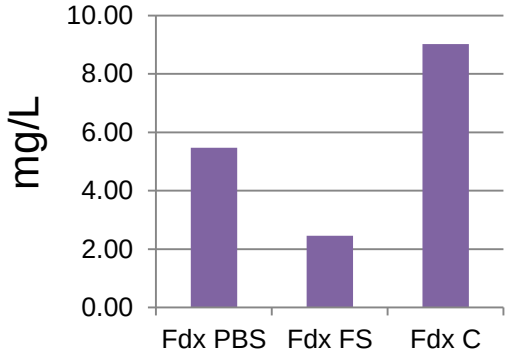
vancomycin metronidazole fidaxomicin surotomycin

	VAN	VAN	VAN	VAN	MET	MET	MET	MET	FDX	FDX	FDX	FDX	SURO	SURO	SURO	SURO
	AI	PBS	FS	C	AI	PBS	FS	C	AI	PBS	FS	C	AI	PBS	FS	C
n=	30	27	27	26	30	27	27	27	30	28	28	27	30	28	28	27
GeoM	0.87	0.54	0.84	0.53	0.33	0.35	38.3	0.34	0.05	0.13	1.5	0.05	0.16	0.30	15.6	0.31

Fold change 1.6 112 30 50





Separation by faeces

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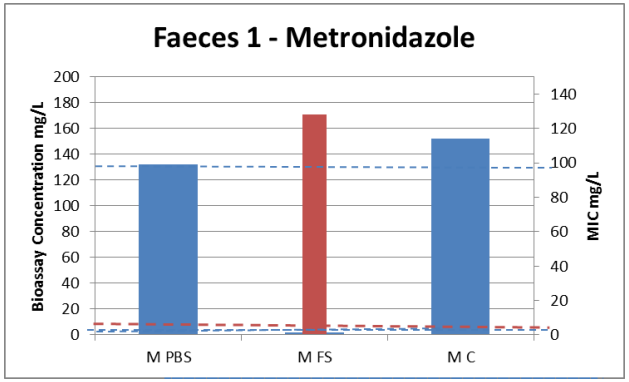

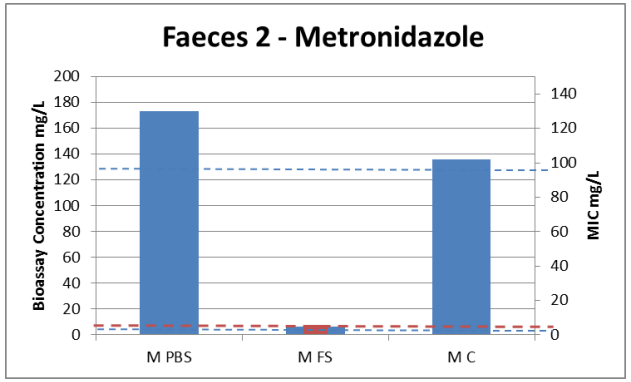

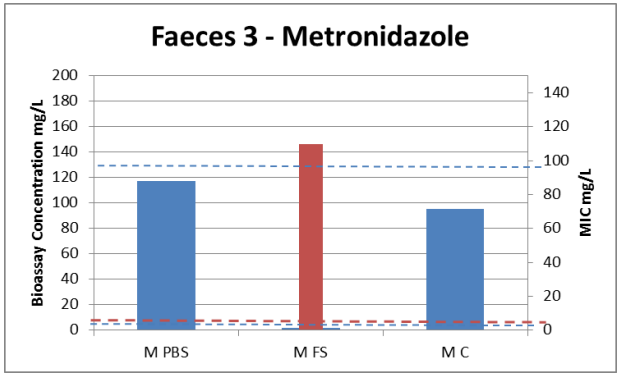

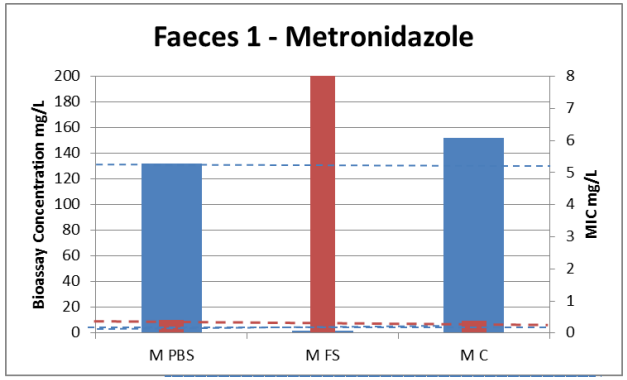

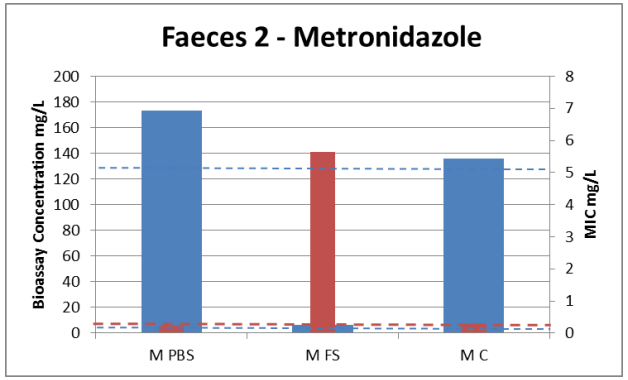

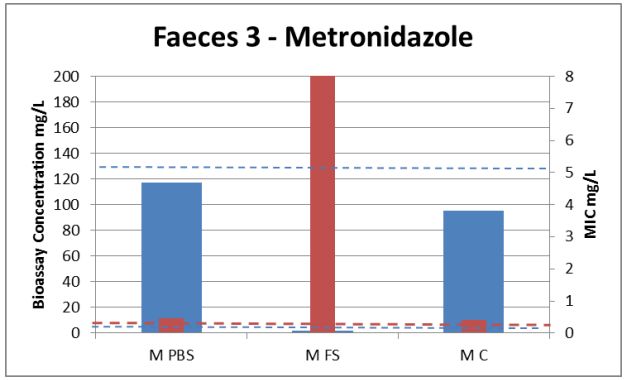

Bioassay	76	22	47	Fold change
MIC	332	21	276	

Separation by faeces

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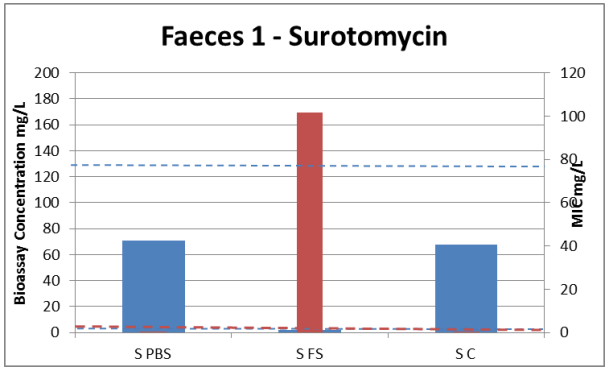

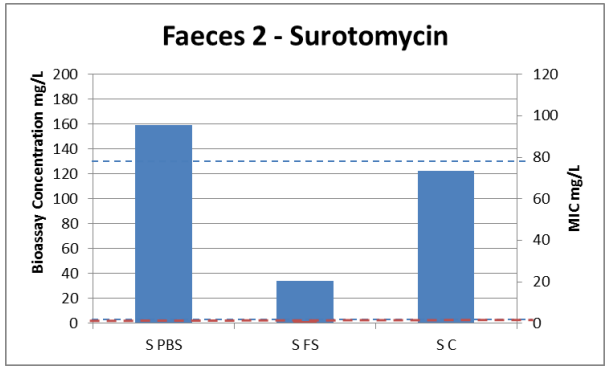

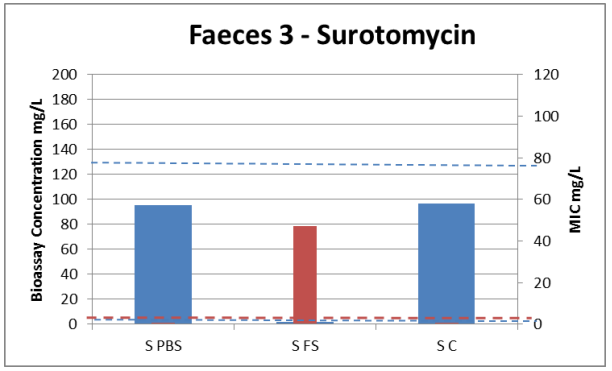

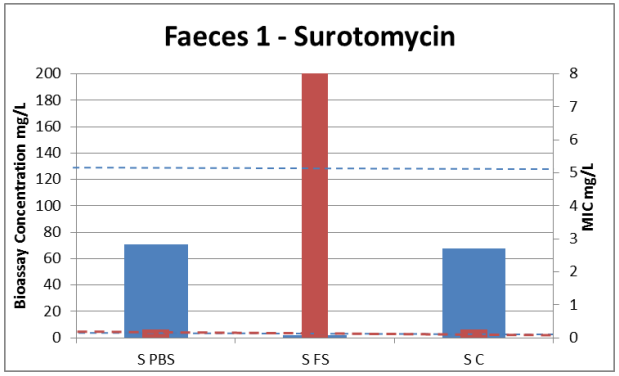

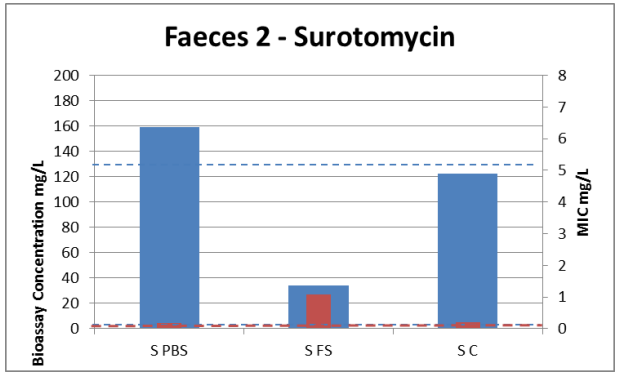

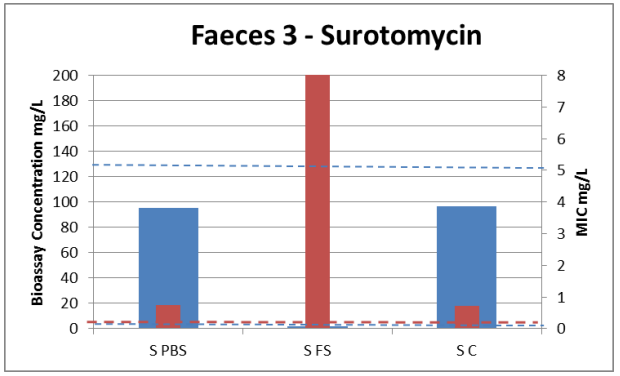

Bioassay	30	3.6	47	Fold Change
MIC	408	5.7	67	

Separation by faeces

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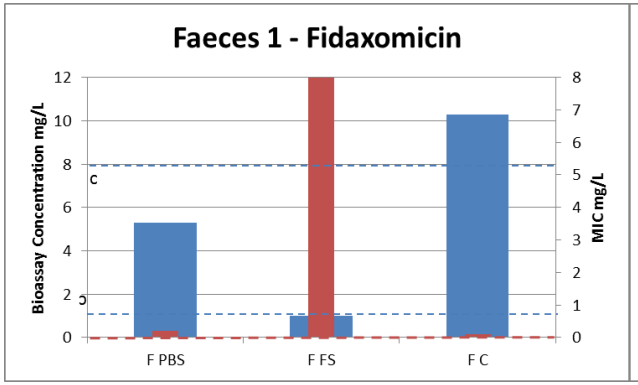

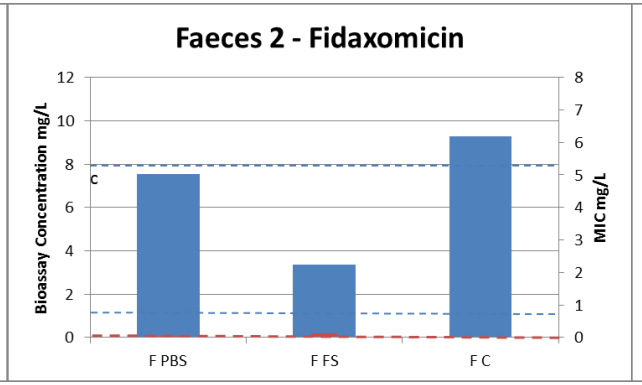

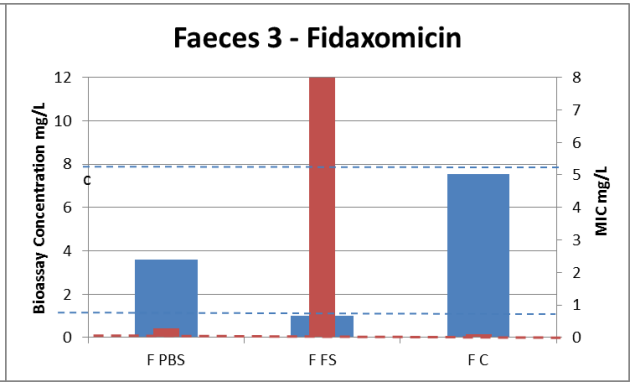

Bioassay	10	2.8	7.5	Fold change
MIC	93	7.9	89	

Separation by faeces

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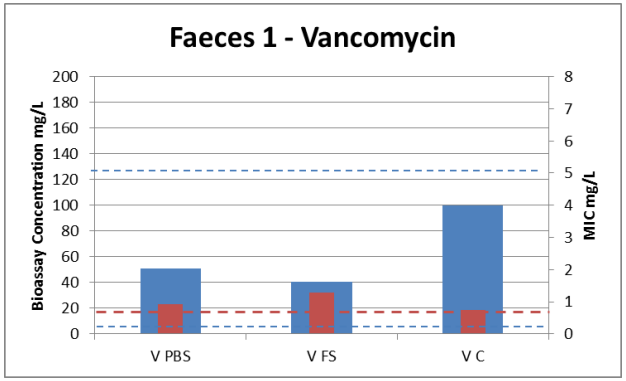

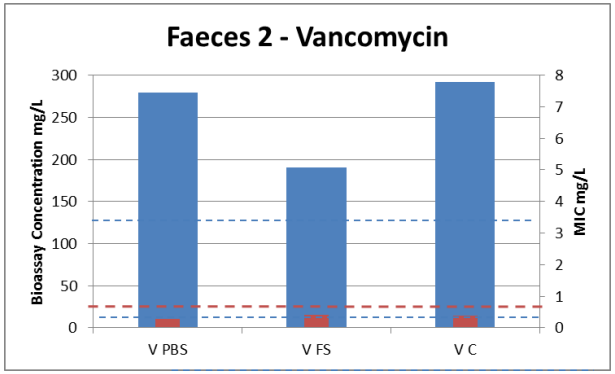

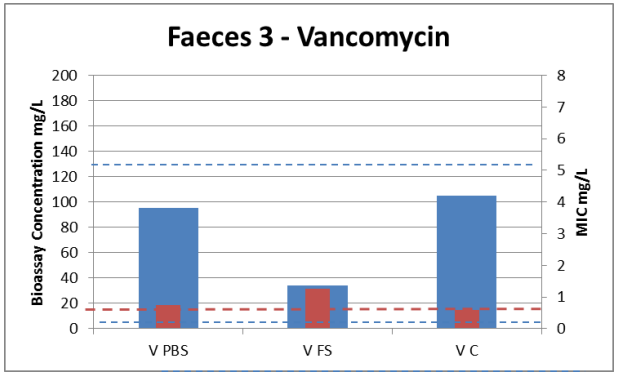

Bioassay	2.4	1.5	3.1	Fold change
MIC	1.7	1.1	2.2	

Questions…

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- What is the mechanism of inactivation?
  - faecal binding
  - enzymatic activity
  - Partition / solubility

  Faecal slurry vs faecal filtrate vs autoclaved samples
- What is the range of variation in different faeces?

  Increased faecal samples. Sequential faecal samples
- Can inactivation be linked to specific microflora populations?

Microflora enumeration

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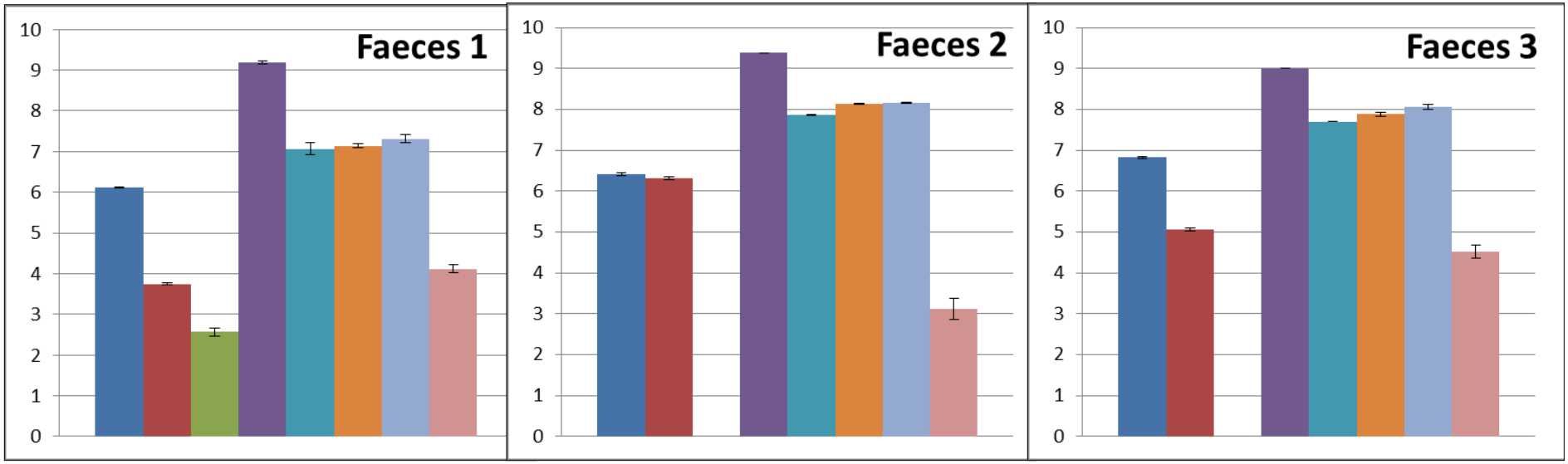

Culture on selective and non-selective agar

- Facultative anaerobes
- Lactose fermenters
- Enterococci
- Total anaerobes
- Clostridia
- *B. fragilis* gp
- Bifidobacteria
- Lactobacilli

Conclusions

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- Incubation with faeces affects detectable drug activity
  - Most pronounced for metronidazole (37-fold) and surotomycin (7.5 fold) compared to vancomycin (2.1-fold) and fidaxomicin (3.7-fold)
- Therefore affects apparent MIC values (inverse relationship)
  - Metronidazole (112-fold), surotomycin (50-fold), vancomycin (1.5-fold), fidaxomicin (30-fold)
- Large variation in affect of different faeces
  - Possibly linked to microflora diversity?
- In some individuals, activity of CDI agents in the colon may be markedly reduced, potentially impacting on treatment success.

METRONIDAZOLE

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danke
Kiitos