Impact of FMT Delivery Modality and Patient Features on Treatment Response in Severe *C. difficile* infection

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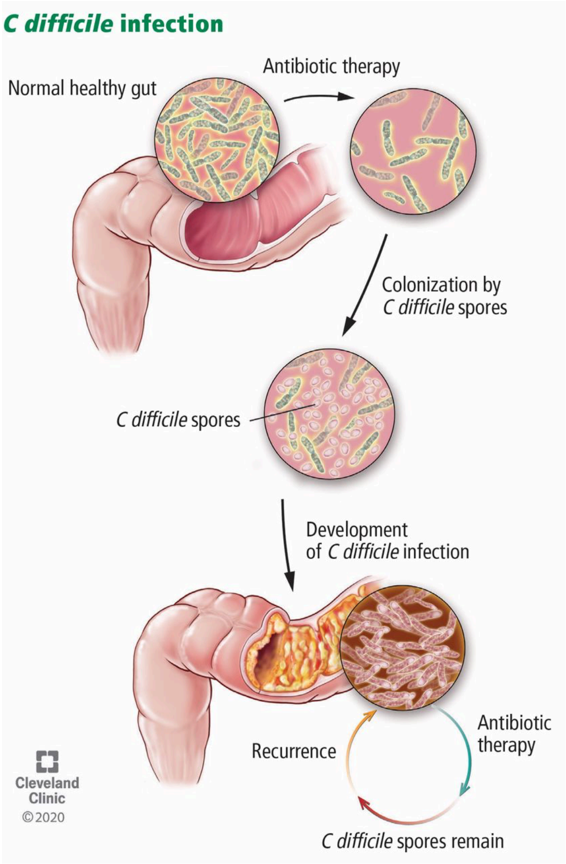
Penn Medicine

Introduction - What is *C. difficile*

- Spore-forming gut bacterium contracted *common* in hospitals but also present in food sources/ agricultural environments
  - Antibiotics clear “good” bacteria → *C. diff* blooms.
- C. Diff releases toxins (tcdA and tcdB)
  - Toxins inflame colon → watery diarrhea ± severe colitis.
- High-risk: recent antibiotics, age 65+, long hospital/LTC stay, weak immunity, antacids (PPIs, H_2 Blockers). (AJG)
  - CKD and ERSD are considered high risk factors → high antibiotics exposure (PMC)

Currently Classified as (PMC, JAC):

- **Non-severe & Recurrent** –
  - FMT already well-established to treat recurrent infection
- **Severe**
- **Fulminant (severe-complicated)** – Hypotension / shock, ileus, or toxic megacolon ± lactate rise, perforation, or multiorgan failure; surgical consult urgent.



https://my.clevelandclinic.org/health/diseases/15548-c-diff-infection

Current Interventions for *C. difficile*

Prevention of SURGERY

1st line of treatment: Targeted Oral Antibiotics

Fidaxomicin (preferred) or vancomycin eradicate toxigenic *C. diff* while sparing much of the microbiome (Nature Med)

~~Immunotherapy~~

~~Monoclonal antibodies against *C. diff* toxin (bezlotoxumab)~~

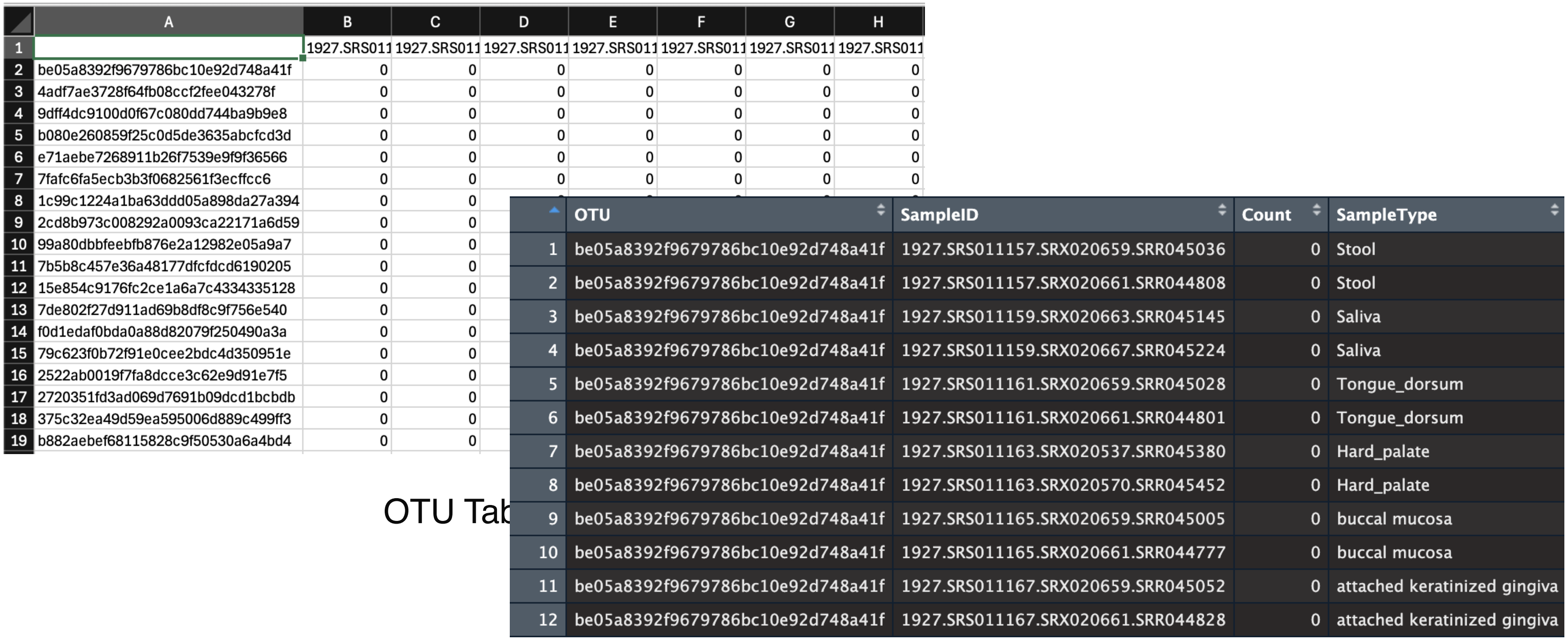
FMT (or other LBPs)

Enteric Tube (Upper GI) and Enema/ Colonscopic (Lower GI)
Now 2 FDA approved versions for **recurrent CDI**
But not certain if effective in severe/fulminant CDI

Measurement and Statistical Model For Microbiome Data

Focus on Beta Diversity (Sorensen) and PCoA

Microbiome Data - HMP Example



	A	B	C	D	E	F	G	H
1		1927.SRS011	1927.SRS011	1927.SRS011	1927.SRS011	1927.SRS011	1927.SRS011	1927.SRS011
2	be05a8392f9679786bc10e92d748a41f	0	0	0	0	0	0	0
3	4adf7ae3728f64fb08ccf2fee043278f	0	0	0	0	0	0	0
4	9dff4dc9100d0f67c080dd744ba9b9e8	0	0	0	0	0	0	0
5	b080e260859f25c0d5de3635abcfcd3d	0	0	0	0	0	0	0
6	e71aebe7268911b26f7539e9f9f36566	0	0	0	0	0	0	0
7	7fafc6fa5ecb3b3f0682561f3ecffcc6	0	0	0	0	0	0	0
8	1c99c1224a1ba63ddd05a898da27a394	0	0					
9	2cd8b973c008292a0093ca22171a6d59	0	0					
10	99a80dbbfeebfb876e2a12982e05a9a7	0	0					
11	7b5b8c457e36a48177dfcfdcd6190205	0	0					
12	15e854c9176fc2ce1a6a7c4334335128	0	0					
13	7de802f27d911ad69b8df8c9f756e540	0	0					
14	f0d1edaf0bda0a88d82079f250490a3a	0	0					
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16	2522ab0019f7fa8dcce3c62e9d91e7f5	0	0					
17	2720351fd3ad069d7691b09dcd1bcbdb	0	0					
18	375c32ea49d59ea595006d889c499ff3	0	0					
19	b882aebef68115828c9f50530a6a4bd4	0	0					

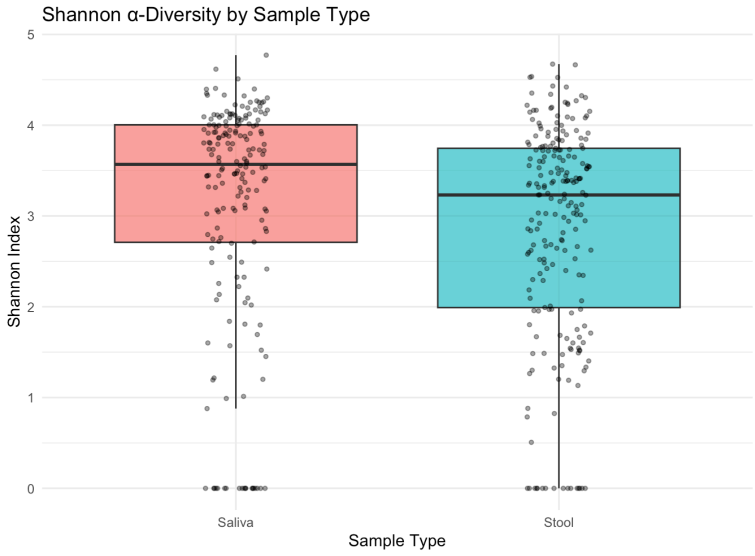
OTU Tab

	OTU	SampleID	Count	SampleType
1	be05a8392f9679786bc10e92d748a41f	1927.SRS011157.SRX020659.SRR045036	0	Stool
2	be05a8392f9679786bc10e92d748a41f	1927.SRS011157.SRX020661.SRR044808	0	Stool
3	be05a8392f9679786bc10e92d748a41f	1927.SRS011159.SRX020663.SRR045145	0	Saliva
4	be05a8392f9679786bc10e92d748a41f	1927.SRS011159.SRX020667.SRR045224	0	Saliva
5	be05a8392f9679786bc10e92d748a41f	1927.SRS011161.SRX020659.SRR045028	0	Tongue_dorsum
6	be05a8392f9679786bc10e92d748a41f	1927.SRS011161.SRX020661.SRR044801	0	Tongue_dorsum
7	be05a8392f9679786bc10e92d748a41f	1927.SRS011163.SRX020537.SRR045380	0	Hard_palate
8	be05a8392f9679786bc10e92d748a41f	1927.SRS011163.SRX020570.SRR045452	0	Hard_palate
9	be05a8392f9679786bc10e92d748a41f	1927.SRS011165.SRX020659.SRR045005	0	buccal mucosa
10	be05a8392f9679786bc10e92d748a41f	1927.SRS011165.SRX020661.SRR044777	0	buccal mucosa
11	be05a8392f9679786bc10e92d748a41f	1927.SRS011167.SRX020659.SRR045052	0	attached keratinized gingiva
12	be05a8392f9679786bc10e92d748a41f	1927.SRS011167.SRX020661.SRR044828	0	attached keratinized gingiva

Microbiome Diversity Metrics

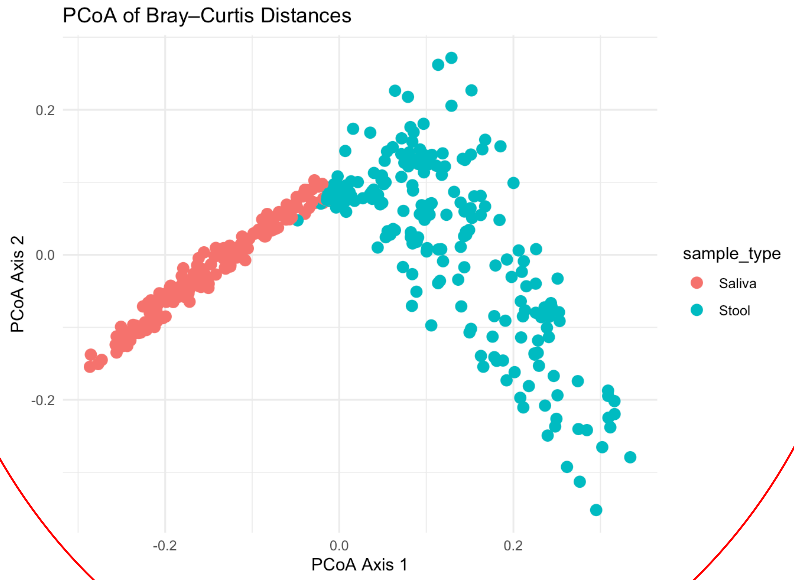
Alpha Diversity

Richness + evenness **within one sample** (e.g., Shannon index).



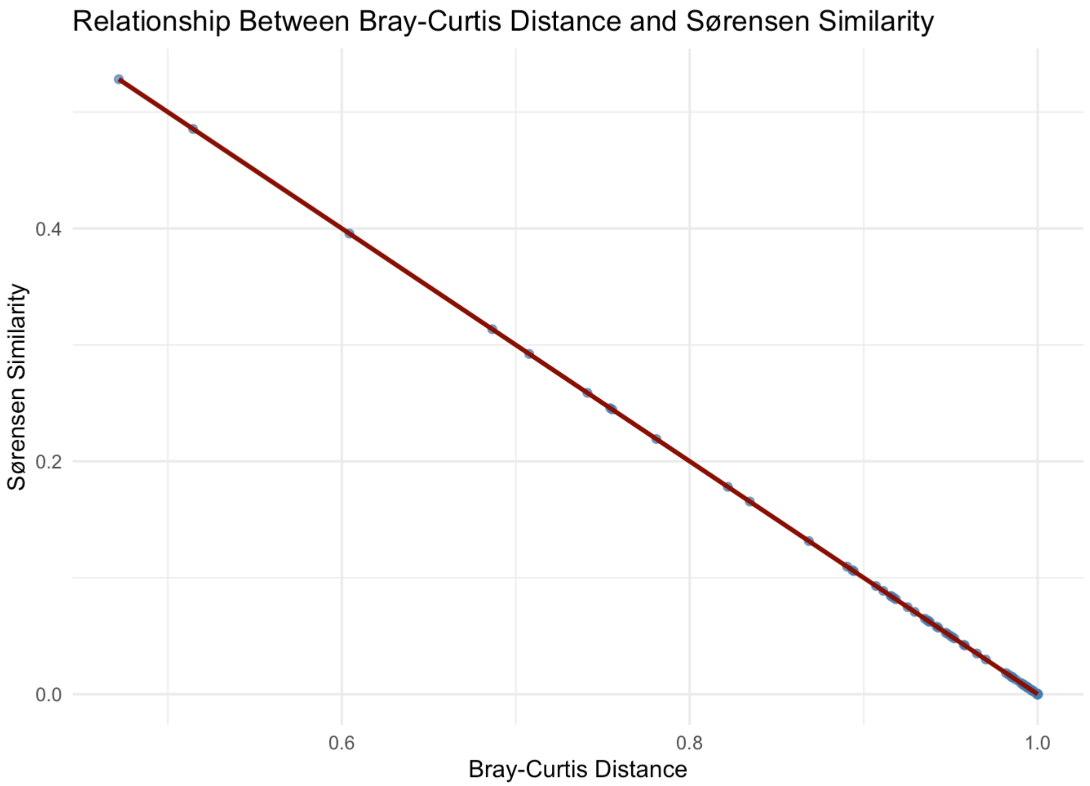
Beta Diversity

Compositional **distance between samples** (e.g., Bray–Curtis or Sorenson).



Quantifying Beta-Diversity: Similarity or Difference

- The OUTCOME variable (Bray-Curtis or Sorenson)
  - Bray-Curtis represents difference
  - Sorenson represents similarity



Methods

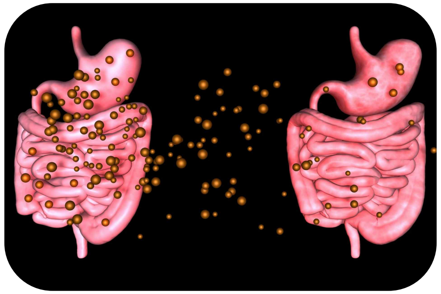
FMT Route (Exposure) → Engraftment Sorenson Values (Outcome)

Aim of the Trial: Clinical Response

Understand and optimize FMT to improve recovery in patients with severe *C. difficile* infection.

Aim of My Study - Microbiome Response

Analyze how the delivery route may influence treatment response, focusing particularly on Beta diversity as outcomes. This could help identify optimal delivery methods and donor characteristics for high-risk *C. difficile* patients, ultimately avoiding life-threatening surgeries.



Comparative Efficacy FMT Route Modality

RCT (Randomized Control Trial)

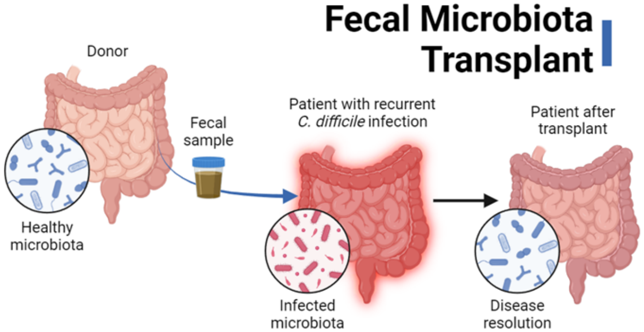
15 subjects
- Randomized first to FMT vs. No FMT (2:1)
- FMT subjects randomized (1:1) Upper vs. Lower

FMT Route

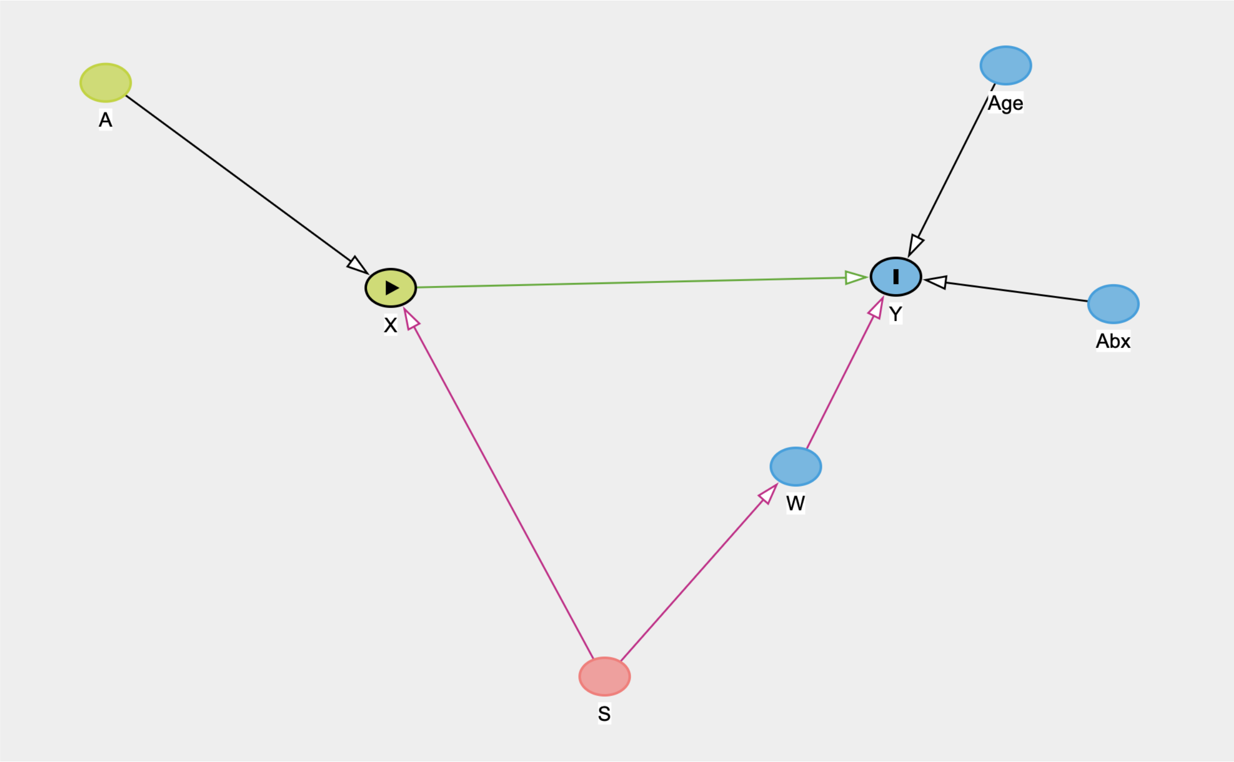
6 subjects received via Lower GI
4 subjected received FMT via Upper GI

FMT vs. No FMT

10 subjects received FMT
5 subjects did not



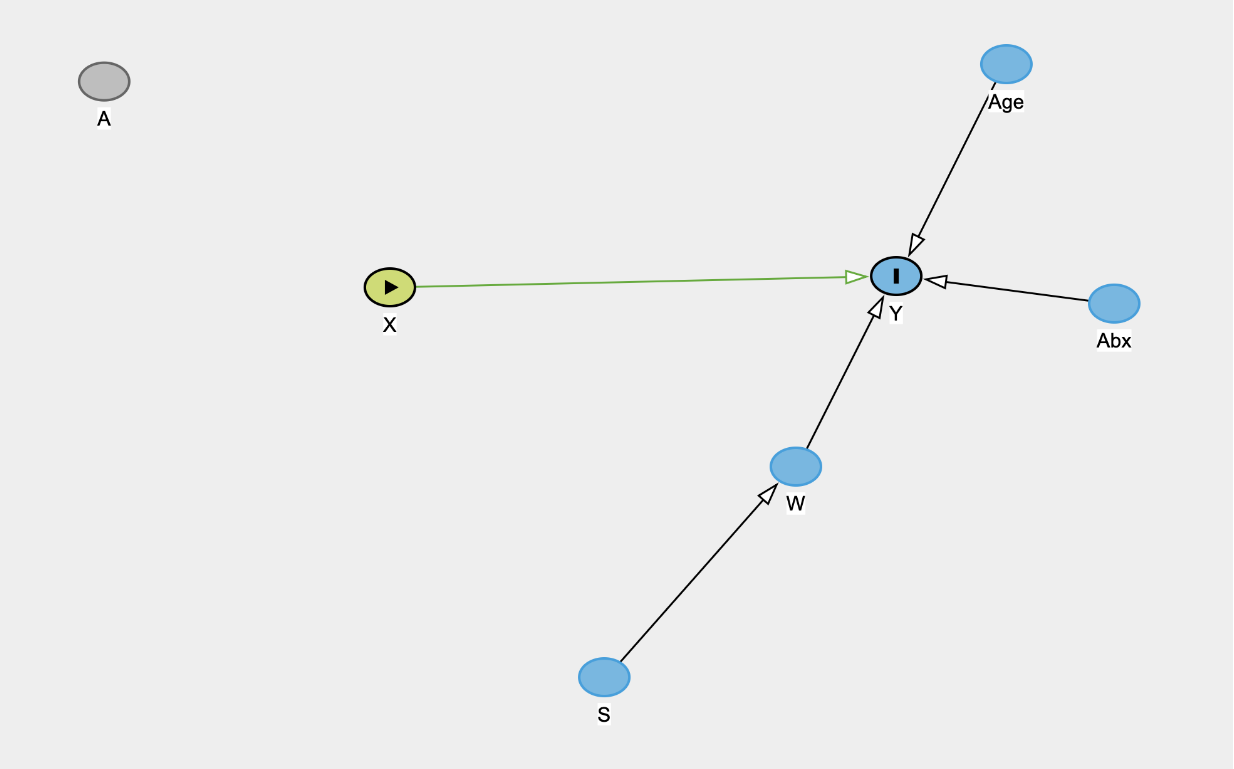
Observational Study-Based DAG



Key:

X (exposure) → FMT Route
Y (outcome) → Engraftment via Beta Diversity
A → Acid Reflux
S → Severity
W → Wipeout of Microbiome
Abx → Antibiotics
Age → Age

RCT-Based DAG



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X (exposure) → FMT Route
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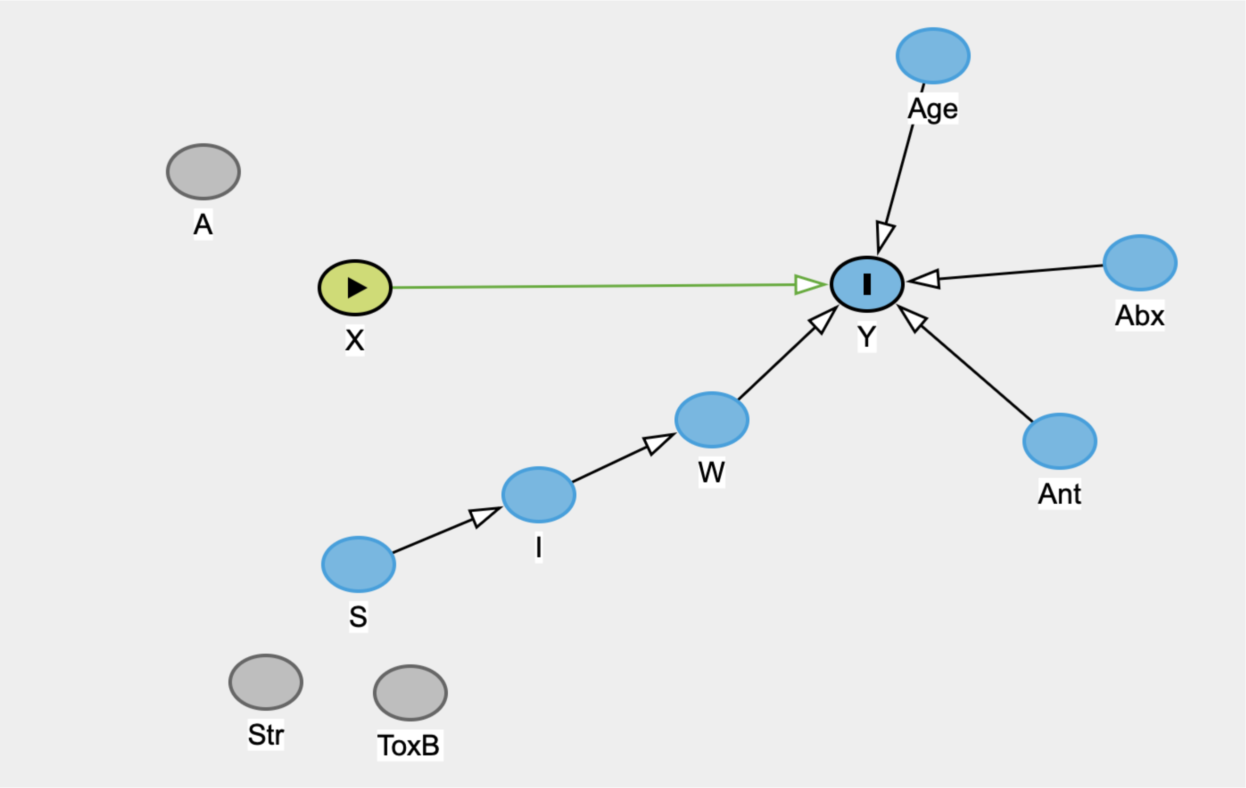
Final DAG

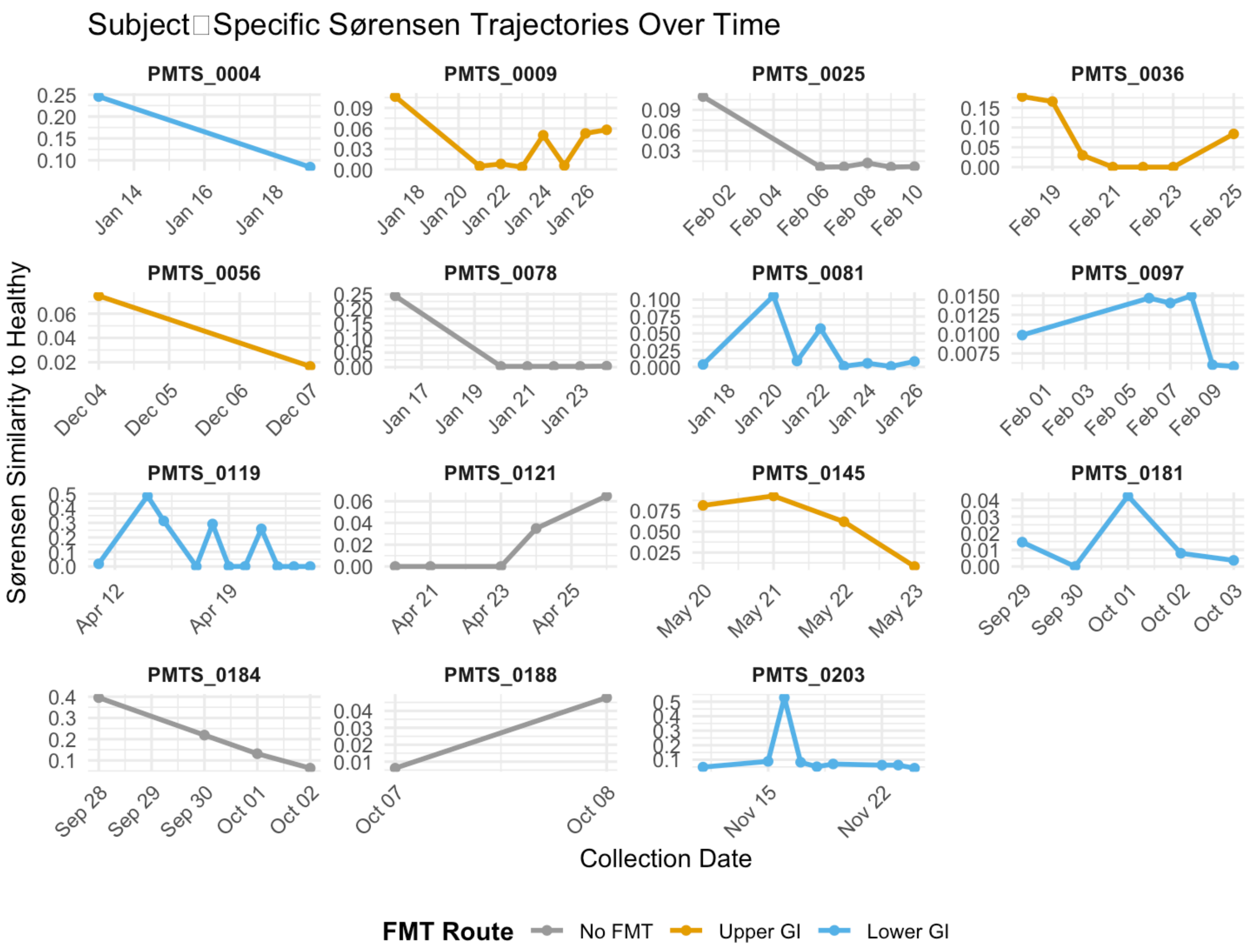


Key:

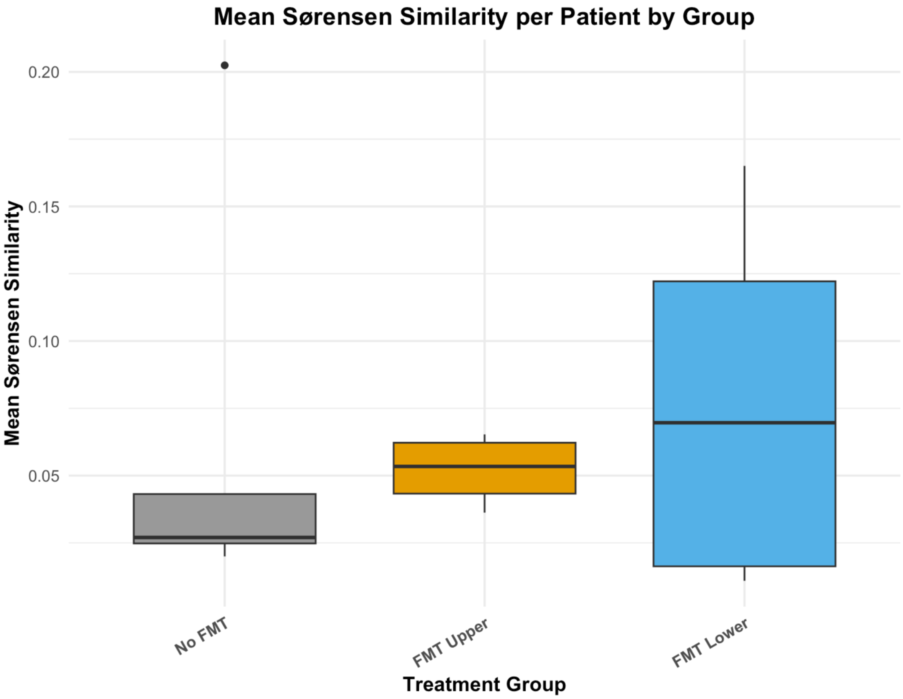
X (exposure) → FMT Route
Y (outcome) → Engraftment via Beta Diversity
A → Acid Reflux
S → Severity
W → Wipeout of Microbiome
Abx → Antibiotics
Age → Age
I → Inflammation
Ant → Antacids

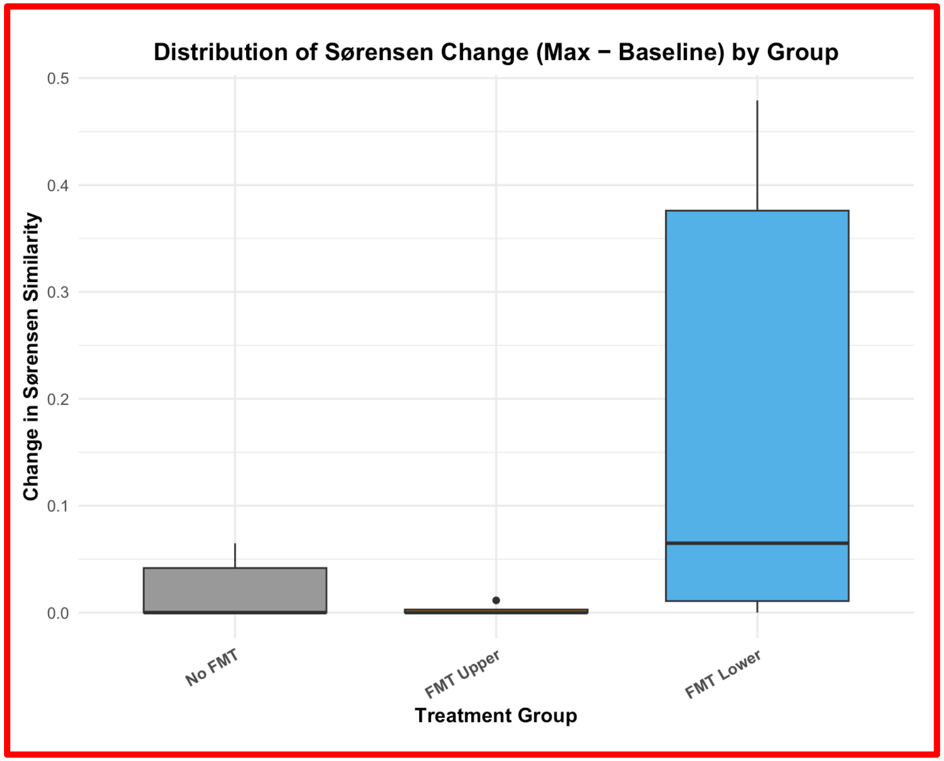
Results

Subject-Specific Sorensen Trajectories

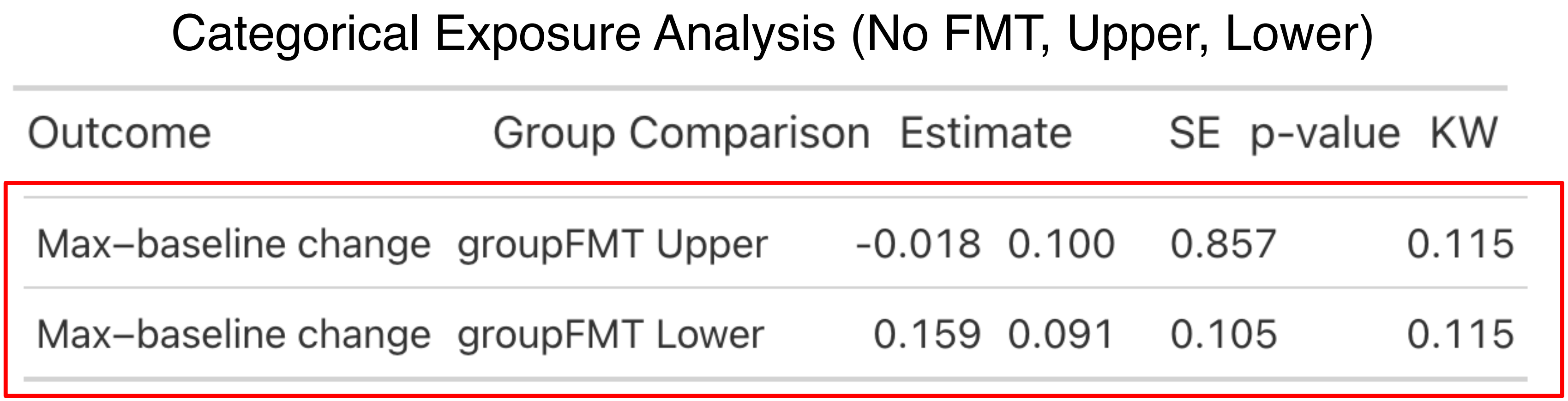


Sorensen Similaritiy Boxplots





Univariable Analysis (Categorical FMT vs. No FMT)



Categorical Exposure Analysis (No FMT, Upper, Lower)

Outcome	Group Comparison	Estimate	SE	p-value	KW
Max–baseline change	groupFMT Upper	-0.018	0.100	0.857	0.115
Max–baseline change	groupFMT Lower	0.159	0.091	0.105	0.115

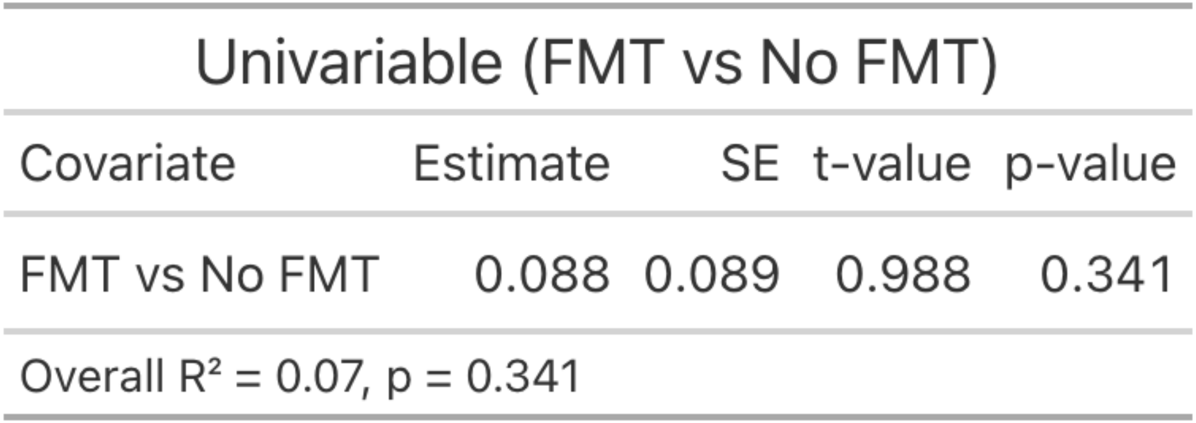
Interpretations:

- Lower GI-Entry → 16% Sorensen increase
- Upper GI-Entry → 0% Sorensen increase

However both did not reach statistical significance

Univariable Analysis (Aggregate) FMT vs. No FMT)

Aggregate Analysis (FMT any vs. No FMT)



Univariable (FMT vs No FMT)

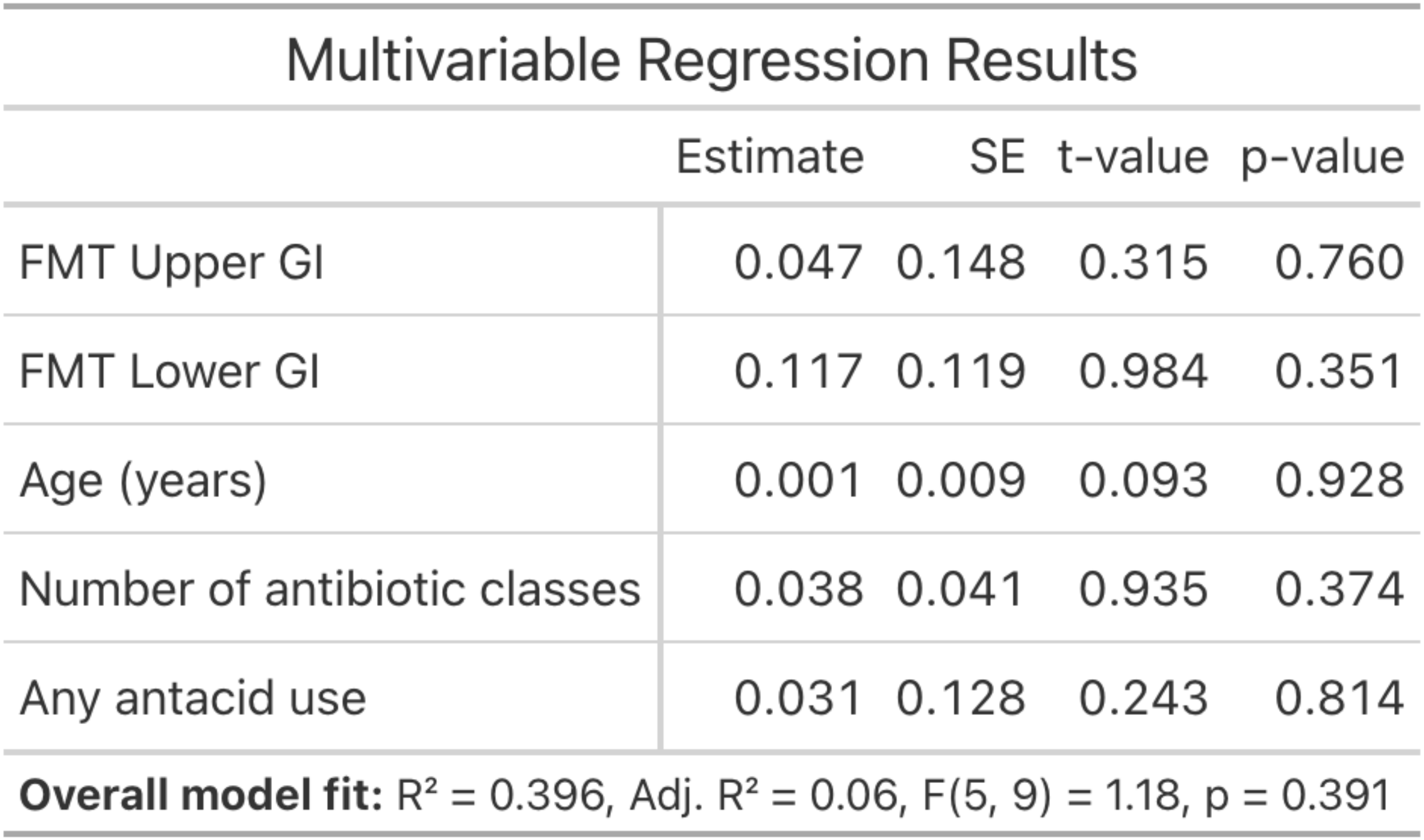
Covariate	Estimate	SE	t-value	p-value
FMT vs No FMT	0.088	0.089	0.988	0.341

Overall $R^2 = 0.07$, $p = 0.341$

Interpretations:

- FMT Any → 9% Sorensen increase
    - Could be attributed to Upper GI-Entry lower change (Higher Baseline Sorensen Value)

Multivariable Regression w/ No FMT as Reference



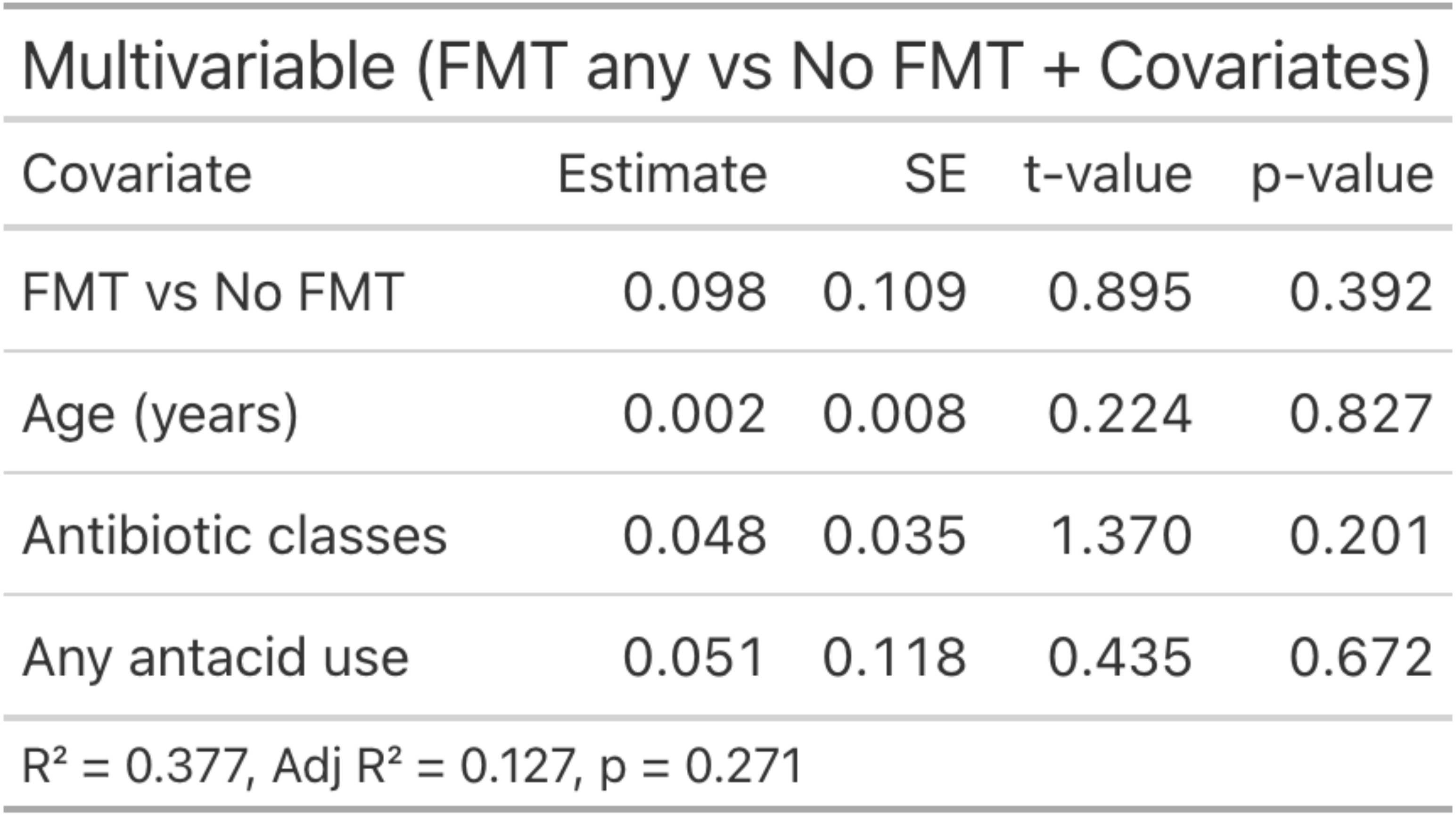
Multivariable Regression Results

	Estimate	SE	t-value	p-value
FMT Upper GI	0.047	0.148	0.315	0.760
FMT Lower GI	0.117	0.119	0.984	0.351
Age (years)	0.001	0.009	0.093	0.928
Number of antibiotic classes	0.038	0.041	0.935	0.374
Any antacid use	0.031	0.128	0.243	0.814

Overall model fit: $R^2 = 0.396$, Adj. $R^2 = 0.06$, $F(5, 9) = 1.18$, $p = 0.391$

No FMT ***Included*** in this regression as the reference value

Multivariable Regression w/ No FMT as Reference



Multivariable (FMT any vs No FMT + Covariates)

Covariate	Estimate	SE	t-value	p-value
FMT vs No FMT	0.098	0.109	0.895	0.392
Age (years)	0.002	0.008	0.224	0.827
Antibiotic classes	0.048	0.035	1.370	0.201
Any antacid use	0.051	0.118	0.435	0.672

$R^2 = 0.377$, Adj $R^2 = 0.127$, $p = 0.271$

Upper AND Lower Aggregated → FMT Any

Conclusion

- Change from baseline in microbiota similarity was comparable between upper-GI and lower-GI FMT.
- Association **greater microbiome recovery** with **lower-GI** delivery, but **not statistically significant**
- Highlights the need for larger, randomized studies.

Limitations

Small Sample Size

Limited ability to detect route-specific effects
Underpowered

External Validity

Single center severe CDI limits generalizability

Residual Confounding

Concurrent antibiotic and antacid use may bias estimates

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Thank you!

Penn Medicine

CCEB

NIH National Institute of Diabetes and Digestive and Kidney Diseases

UCSP Pathway to Clinical Research Careers

Undergraduate Clinical Scholars Program

DBEI