

# Designing Microbiome Trials

## Unique Challenges & Considerations

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# How Randomized Trials Go Wrong

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## RCTs of FMT

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# "Understanding and Misunderstanding RCTs"

- Randomized clinical trials = gold standard for clinical research:
  - balance known & unknown confounders
  - powerful tool to measure causal effects
- RCTs can still mislead:
  - selection bias (subjects ≠ population)
  - "trial interventions are interactions" resulting in heterogeneous effects (subject features modify effect of trial intervention)
  - "subjects aren't even fully representative of themselves" (time-varying features also interact with trial intervention)

# Time-Varying Gut Microbiota

**Table 1.** Effect sizes observed from various exposures/interventions in studies of various microbiome sampling sites are shown as measured by omega-squared ( $\omega^2$ ) statistics, together with the *P*-values from PERMANOVA test

Site	Comparison groups		$\omega^2/P$ -value				Reference
	Control	Exposure	Weighted UniFrac	Unweighted UniFrac	Weighted Jaccard	Unweighted Jaccard	
Nares	Non-smoker (33)	Smoker (29)	0.042/0.001	0.009/0.001	0.023/0.001	0.007/0.001	Charlson <i>et al.</i> (2010)
Oral	Non-smoker (33)	Smoker (29)	0.032/0.001	0.008/0.001	0.024/0.001	0.007/0.001	Charlson <i>et al.</i> (2010)
Gut	Before feeding (10)	After feeding (10)	0.056/0.138	0.013/0.986	0/0.989	0.014/0.985	Wu <i>et al.</i> (2011)
Oral	No azithromycin (42)	Azithromycin (6)	0.063/0.01	0.039/0.001	0.099/0.004	0.032/0.001	Charlson <i>et al.</i> (2012)
Lung	No azithromycin (34)	Azithromycin (6)	0.065/0.005	0.038/0.001	0.019/0.089	0.033/0.001	Charlson <i>et al.</i> (2012)
Skin	Left retroauricular (186)	Right retroauricular (187)	0.000/0.828	0.0001/0.327	0.000/0.986	0.000/1.000	HMP Consortium (2012b)
Human	Anterior nares (161)	Stool (187)	0.567/0.001	0.201/0.001	0.230/0.001	0.117/0.001	HMP Consortium (2012b)

The range of observed effect sizes differs according to the metric of pairwise distance chosen for analysis. HMP data are shown to demonstrate a large effect (the degree of difference between two different human microbiome sampling sites) and a negligible effect (the degree of difference between skin sampling in the left versus right retroauricular crease)

# Time-Varying Gut Microbiota

- Dietary changes and antibiotics exert large effects...  
... larger effects when followed by colonization from healthcare environment
- Gut microbiota at time of intervention ≠ microbiota at time of enrollment:
  - misclassification of inclusion criteria kills randomized trials
  - "the medicine doesn't work if the patient isn't sick"
  - microbiome trials run a **high risk of eligibility misclassification**

# How Microbiota Trials Go Right

- RCTs can overcome effect modification by changing gut microbiota if:
  - very precise disease phenotype (less likely time-varying gut microbiota)
  - precision medicine: tailor treatment to near-real-time measures of gut microbiota
  - interventions with huge effects (antibiotic conditioning prior to FMT)

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## RCTs of FMT

# Strategies for the LBP Era

# Fecal Microbiota Transplant (FMT) Trials

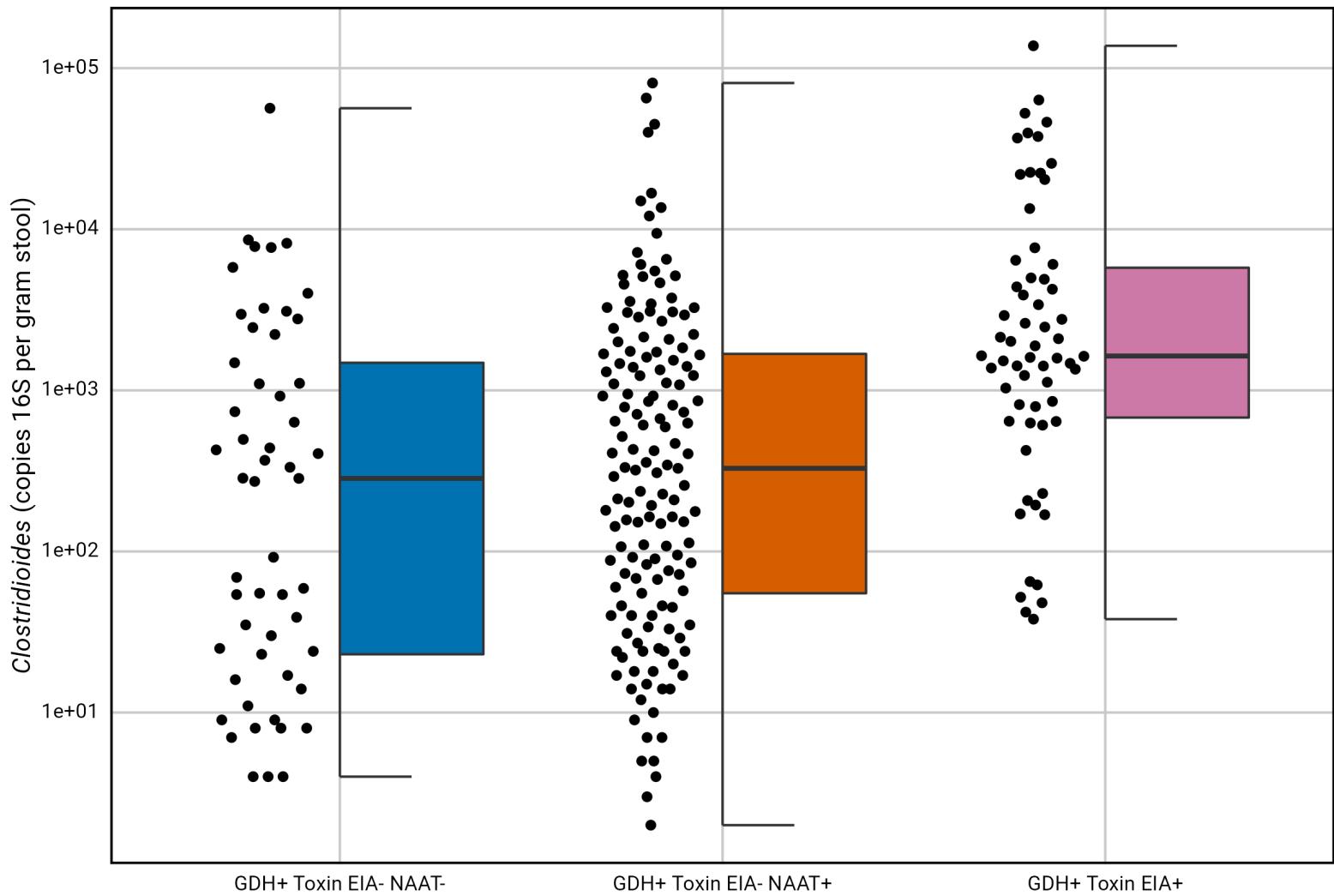
- FMT in Infectious Diseases @ the University of Pennsylvania:
  - FMT dose finding for recurrent *C. difficile* infection (NCT03973697)
  - serial FMT for severe *C. difficile* infection (NCT03970200)
  - FMT for MDRO colonization (CDC sponsored)
- Successful applications of FMT:
  - recurrent & severe *C. difficile*, inflammatory bowel diseases, potentiation of anti-PD1 immunotherapy, MDRO colonization, food allergy mitigation
  - heterogeneity of gut microbiota community composition?

# Microbial Heterogeneity in *C. difficile*

- **Aim:** microbiome features that discriminate *C. difficile* colonization / infection
- **Population:** 384 consecutive positive *C. difficile* tests (in- & outpatient)

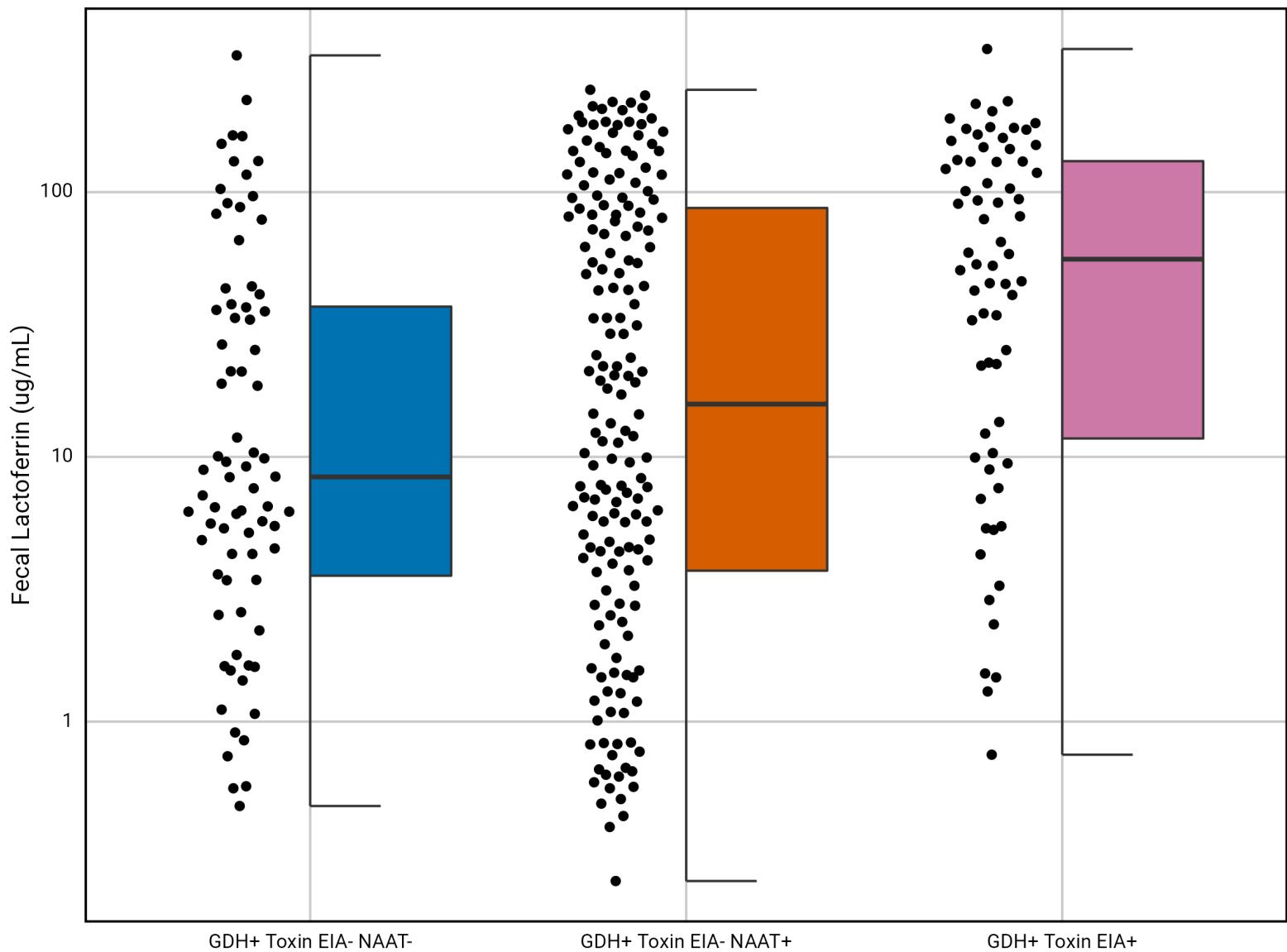
<i>C. difficile</i> Category	Subject Count	Proportion
GDH+ Toxin EIA- NAAT-	94	24.5%
GDH+ Toxin EIA- NAAT+	213	55.5%
GDH+ Toxin EIA+	77	20.1%

- **Sampling:** stool 16S rRNA gene sequencing & 16S rRNA gene qPCR
- **Comparison:** toxin EIA+ (infection) versus NAAT+ only (colonization)
- **Outcome:** EIA+, with fecal lactoferrin as sensitivity analysis



Univariable toxin EIA model: *Clostridioides* OR 1.07 (95%CrI 1.02 - 1.12)

Multivariable toxin EIA model: *Clostridioides* OR 1.05 (95%CrI 1.01 - 1.16) & *Lachnospiraceae* OR 0.32 (95%CrI 0.14 - 0.96)



# Phenotype Definitions & FMT Outcomes

- The success of FMT depends on homogeneity of gut microbiota phenotype:
  - moderate heterogeneity of *C. difficile* infection
  - large heterogeneity across IBD
- Pre-FMT antibiotic conditioning as a method to enforce homogeneity:
  - which antibiotics are used?
  - effects of other medications active on gut microbiota (e.g., PPIs)?
  - FMT as part of a bundled intervention?

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# Into the LBP Era

- Live biotherapeutic products (LBPs) replacing FMT:
  - Ferring, Seres, Vedanta, Finch...
  - ensure greater homogeneity of intervention
  - larger effects?
- Are bundled interventions still necessary?
  - role for pre-LBP antibiotic conditioning?
  - restrictions on medications that reshape gut microbial communities?
  - role for precision medicine & pre-treatment microbiota profiles?

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# Topics for Discussion

- Every RCT intervention is an interaction:
  - gut microbiota community composition varies over time
  - account for changes in diet & medications, environment
- RCTs can overcome effect modification if:
  - very precise disease phenotype (less likely time-varying gut microbiota)
  - precision medicine: tailor treatment to near-real-time measures of gut microbiota
  - interventions with huge effects (antibiotic conditioning prior to FMT)
- How to translate lessons from FMT trials to the LBP era?



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