



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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Strategies for
the LBP Era

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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 (ω^2) statistics, together with the *P*-values from PERMANOVA test

Site	Comparison groups		ω^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 inclusion 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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The Promise of Fecal Microbiota Transplant (FMT)

- How do antibiotics fail?
 - failure of pathogen identification → wrong antibiotic
 - high-grade phenotypic resistance or inadequate source control
 - post-antibiotic repopulation
- Bacterial ecology of infection: VAP, *C. difficile*
- Microbiome change during & after antibiotics
- Engineering post-antibiotic repopulation

Repopulation & Recurrent Infection

- Why does post-antibiotic repopulation matter?
- Failed repopulation risks recurrent infection:
 - healthy subjects time to post-antibiotic repopulation ~ 3 months
 - recurrent *C. difficile* infection (R-CDI): ~ 20% post-treatment recurrence
 - bloodstream, respiratory and urinary tract infections?

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Conclusions



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