How to count pathogens and microbiota during controlled human infections

O. Colin Stine

University of Maryland Baltimore

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Counting is important

- Children in low-income countries often have pathogens, but no diarrheal disease
- **Abundance of each pathogen is critical for disease**
- **Controlled human infections**, “incubation period” is when the pathogen reaches the necessary abundance to cause disease
- Abundance of other bacteria affects whether there is an effect

Four Ways to Count during CHI

Quantitative culture

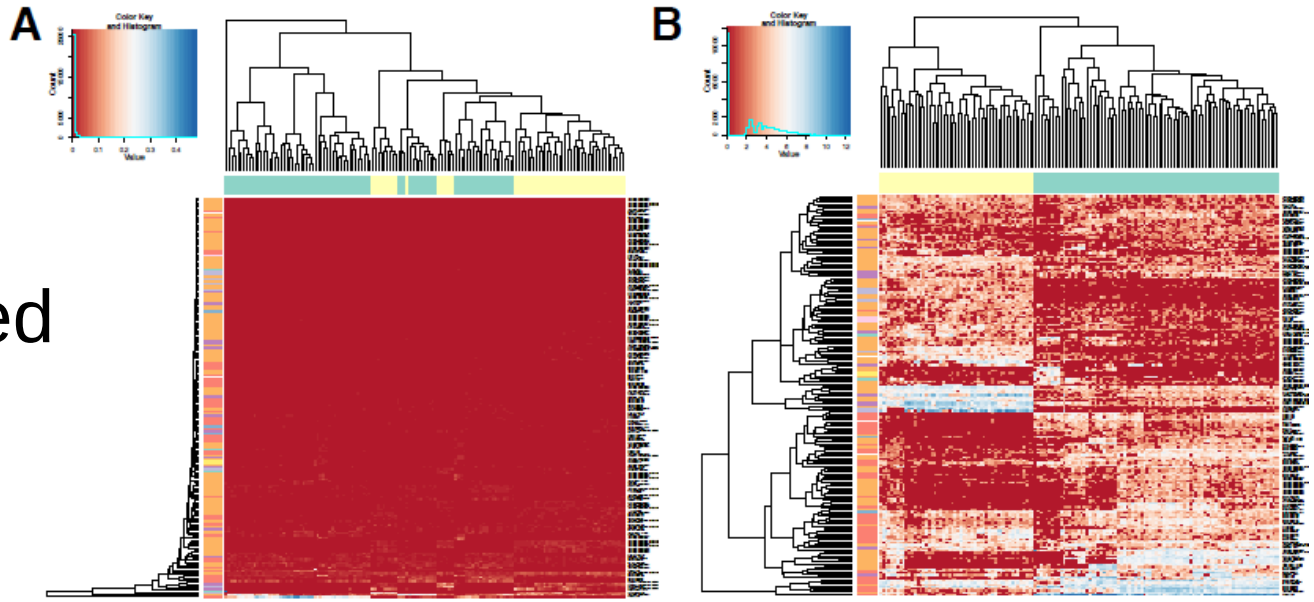
Quantitative (Q)PCR

16S rDNA sequencing

Must be properly normalized

Metagenomic analysis

Normalization of the same data set



Improper

Proper

Paulson Nat Meth 2013

Summary of ETEC trial @ JHU, D. Sack

- 30 Subjects
  - 15 given 10^5 inoculum ETEC H10407
  - 15 given 10^6 inoculum ETEC H10407
- Measurements taken at Day 0 – Day 4
- 9 Subjects developed diarrhea

Subject	Day 0	Day 1	Day 2	Day 3	Day 4
0004					
0009					
0011				★	
0014					
0016				★	
0017					★
0035					
0038				★	
0041					
Total New Cases	**0**	**2**	**3**	**4**	**0**

First	
Subsequent	
Ab given	★

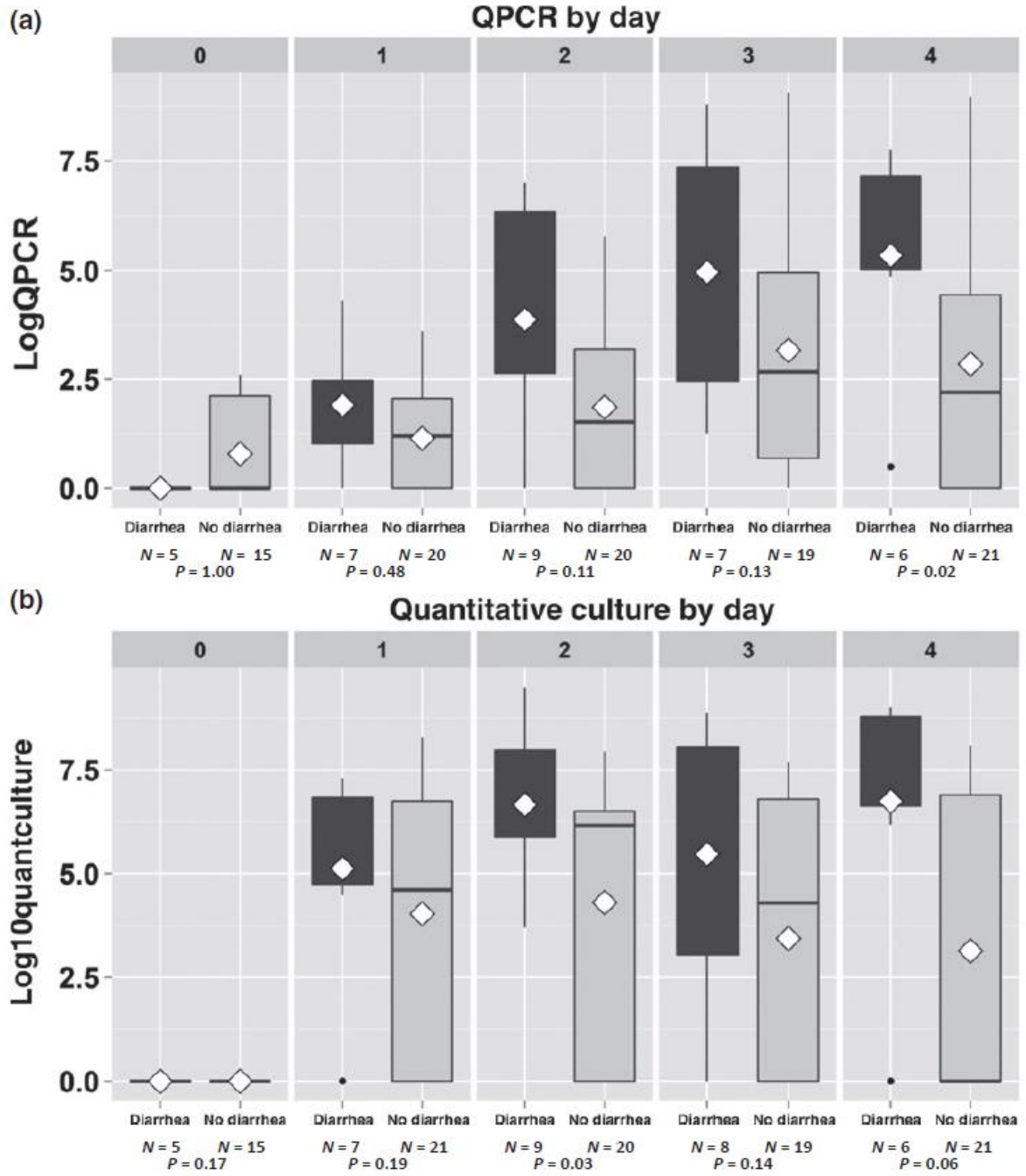
CHI patients who develop diarrhea have with more ETEC

QPCR

vs

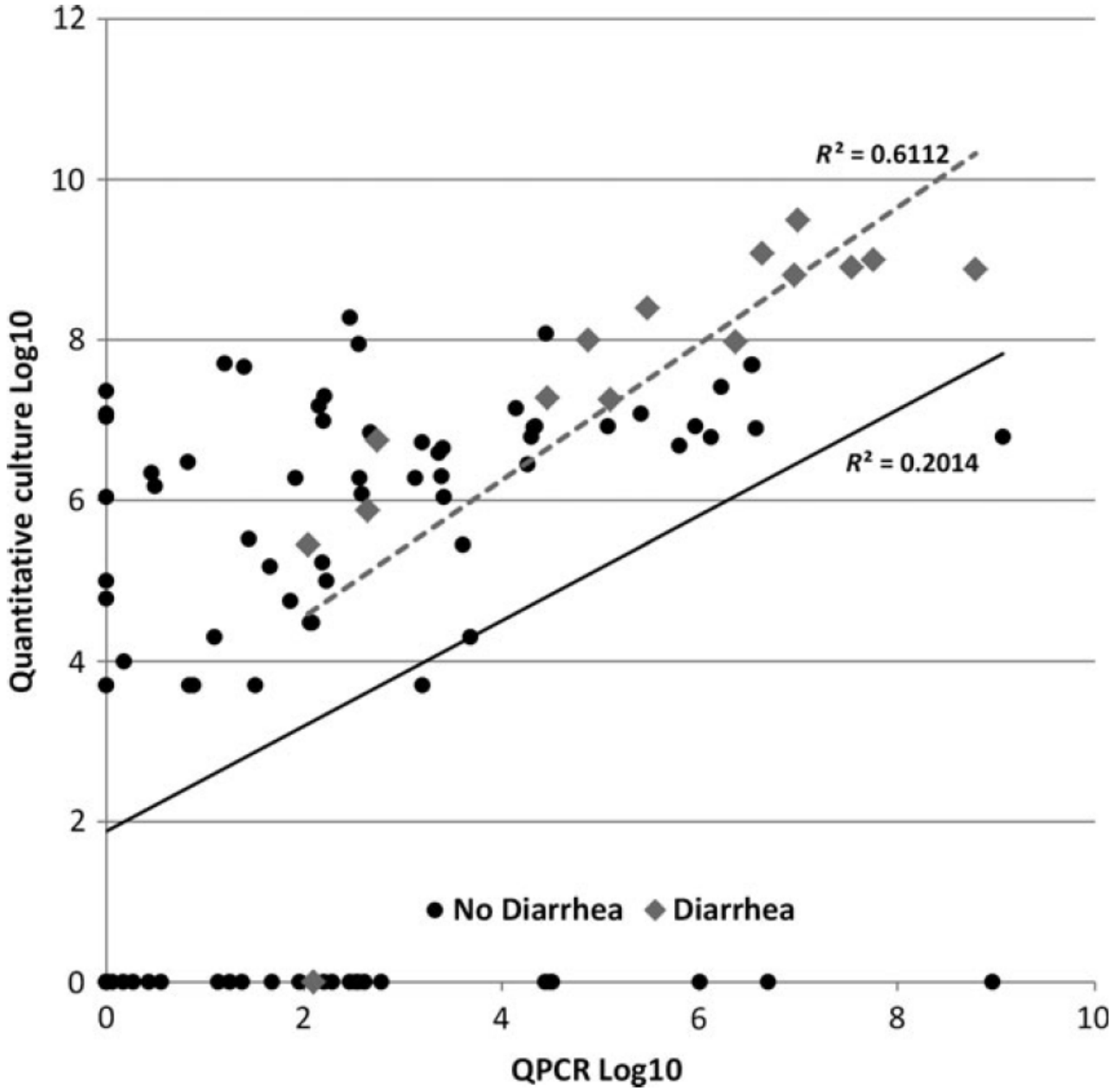
Quantitative culture

Individuals
With diarrhea black
Without grey



Correlation between QPCR and quantitative culture

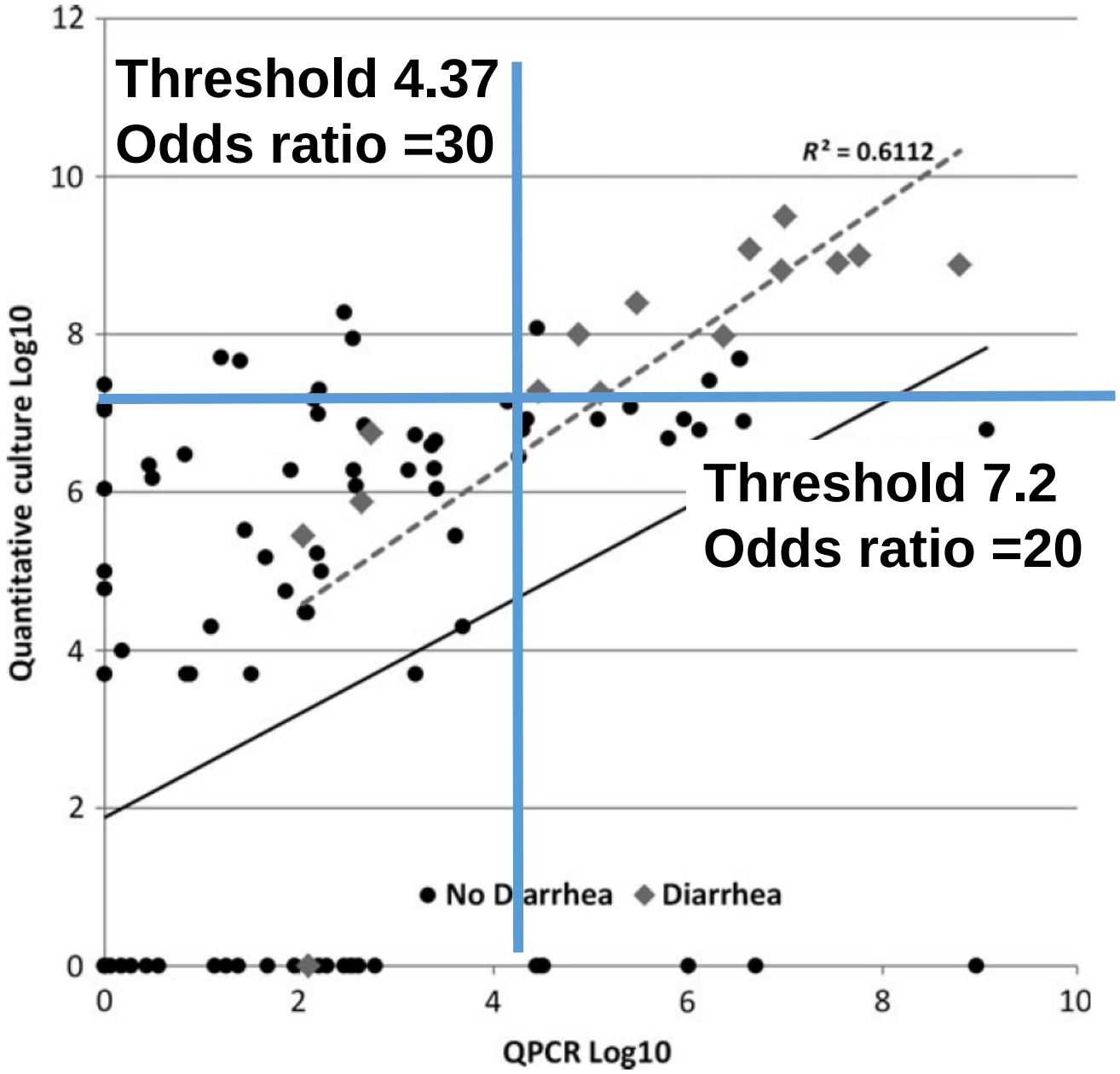
Better when individual has diarrhea (grey diamonds) than does not (black circles)



Thresholds for QPCR and quantitative culture

Limit of detection is below the threshold for ETEC causing disease

Thresholds must be established to predict causality



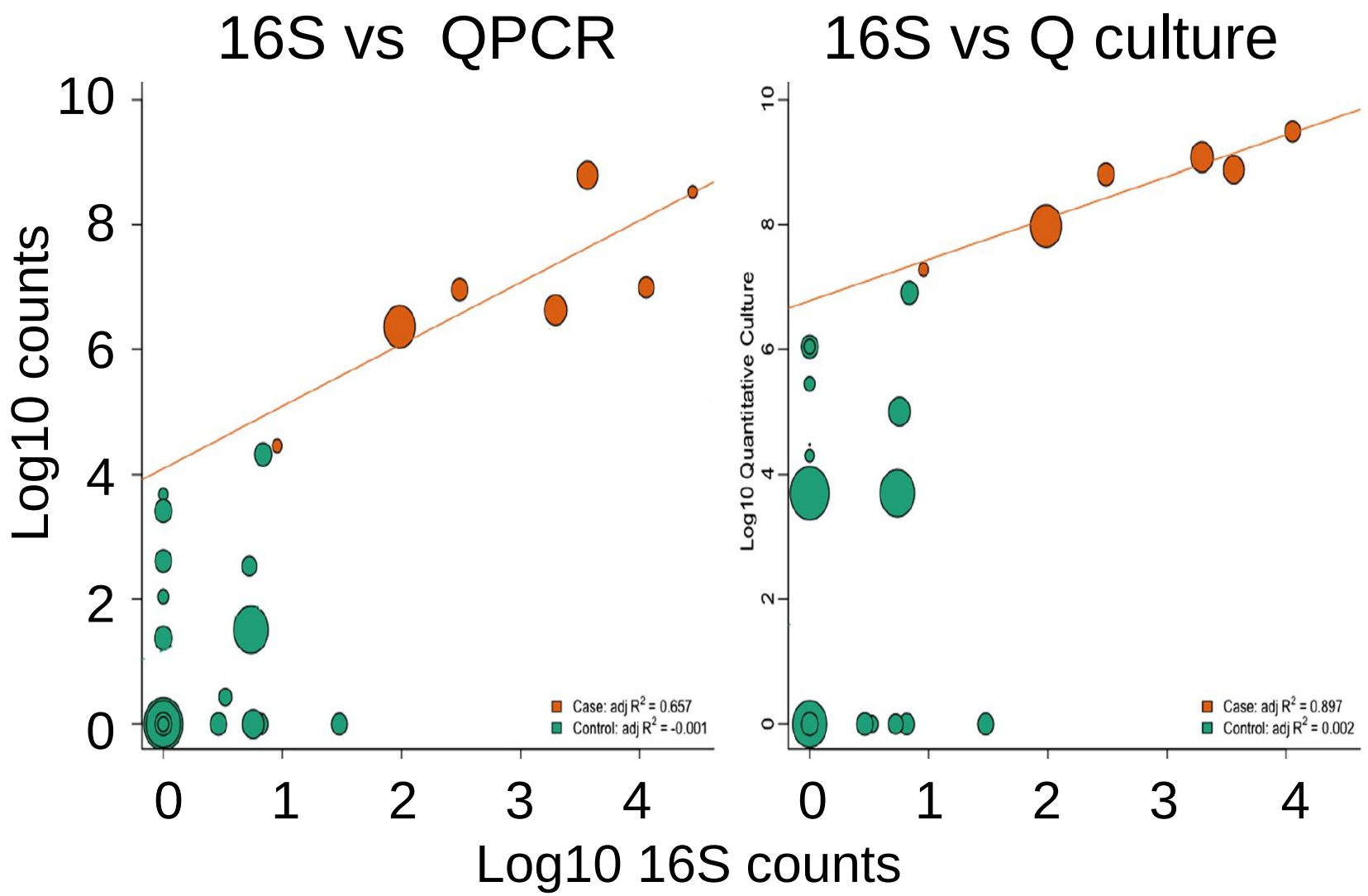
16S *Escherichia* Abundance

In diarrheal samples

16S rRNA gene counts correlate with QPCR or Q culture

But not in non-diarrheal samples.

Limit of detection for 16S: 1 in 6000 bacteria

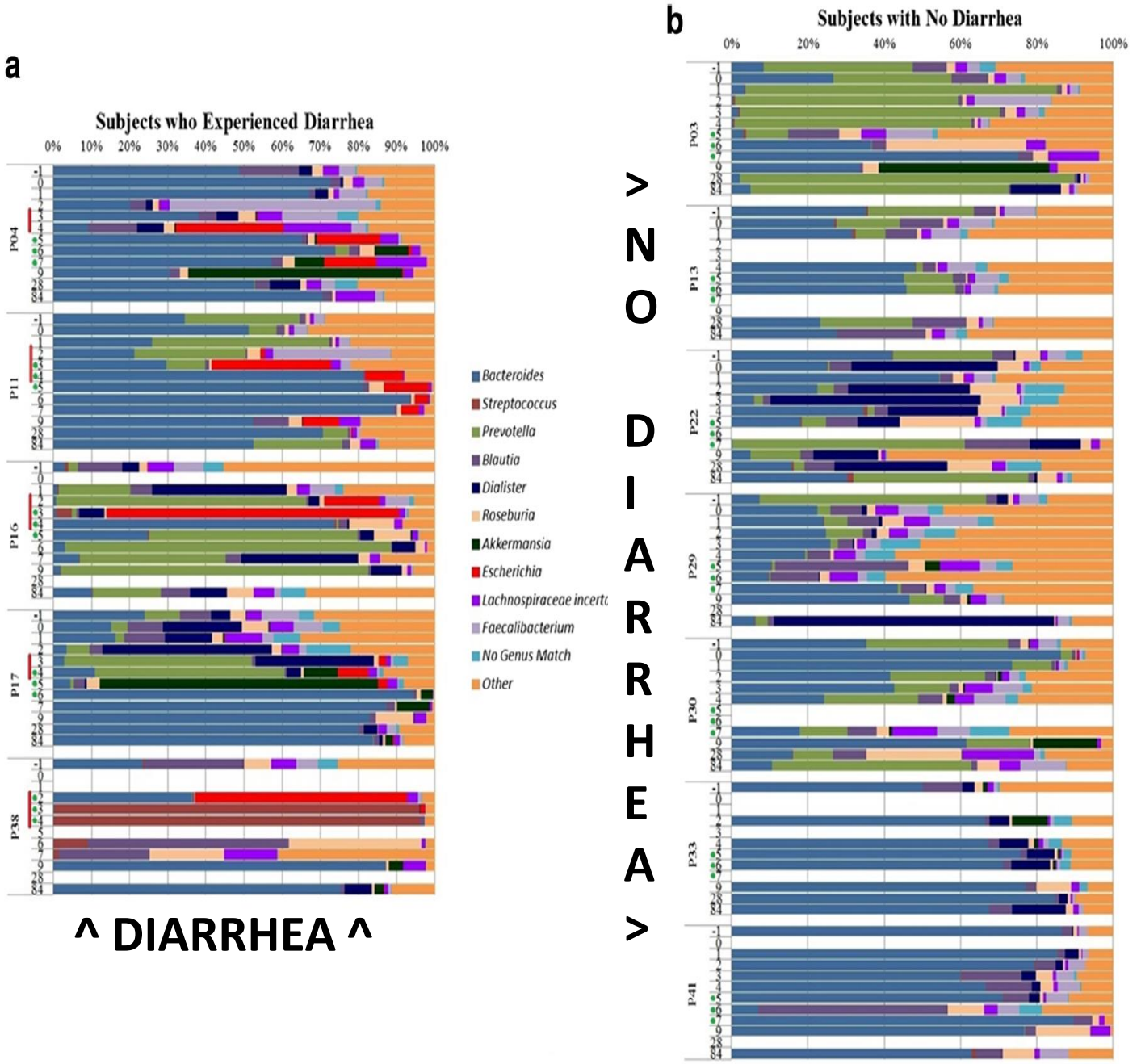


Proportion of top ten genera

Bacteroides most common (blue) Prevotella (green)

Escherichia (**red**) linked to the clinical observations.

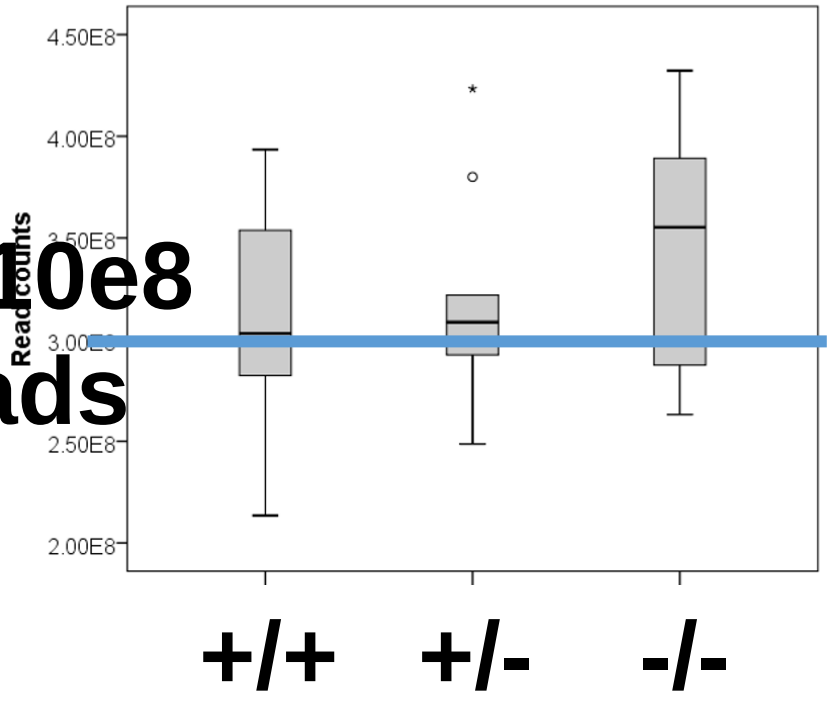
Substantial individual variation



Metagenomic sequencing

Sequence DNA at random

Enormous quantities of data

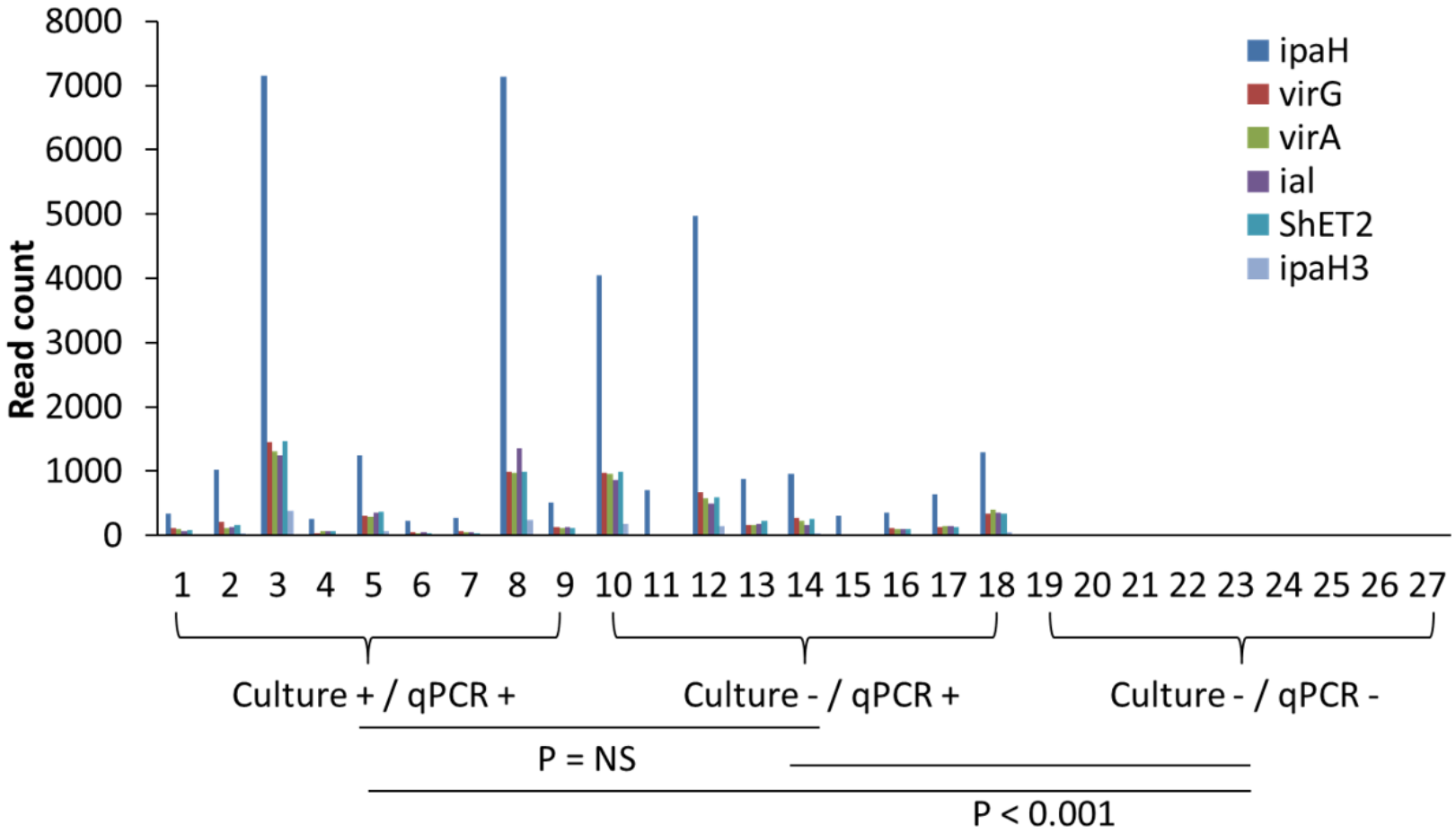


27 samples: 9 QPCR+ culture+, 9 QPCR+ culture-, 9 QPCR- culture-

Which are QPCR+ culture- more like?

Count only genes used for identification of *Shigella*

QPCR+Culture- more like QPCR+culture+

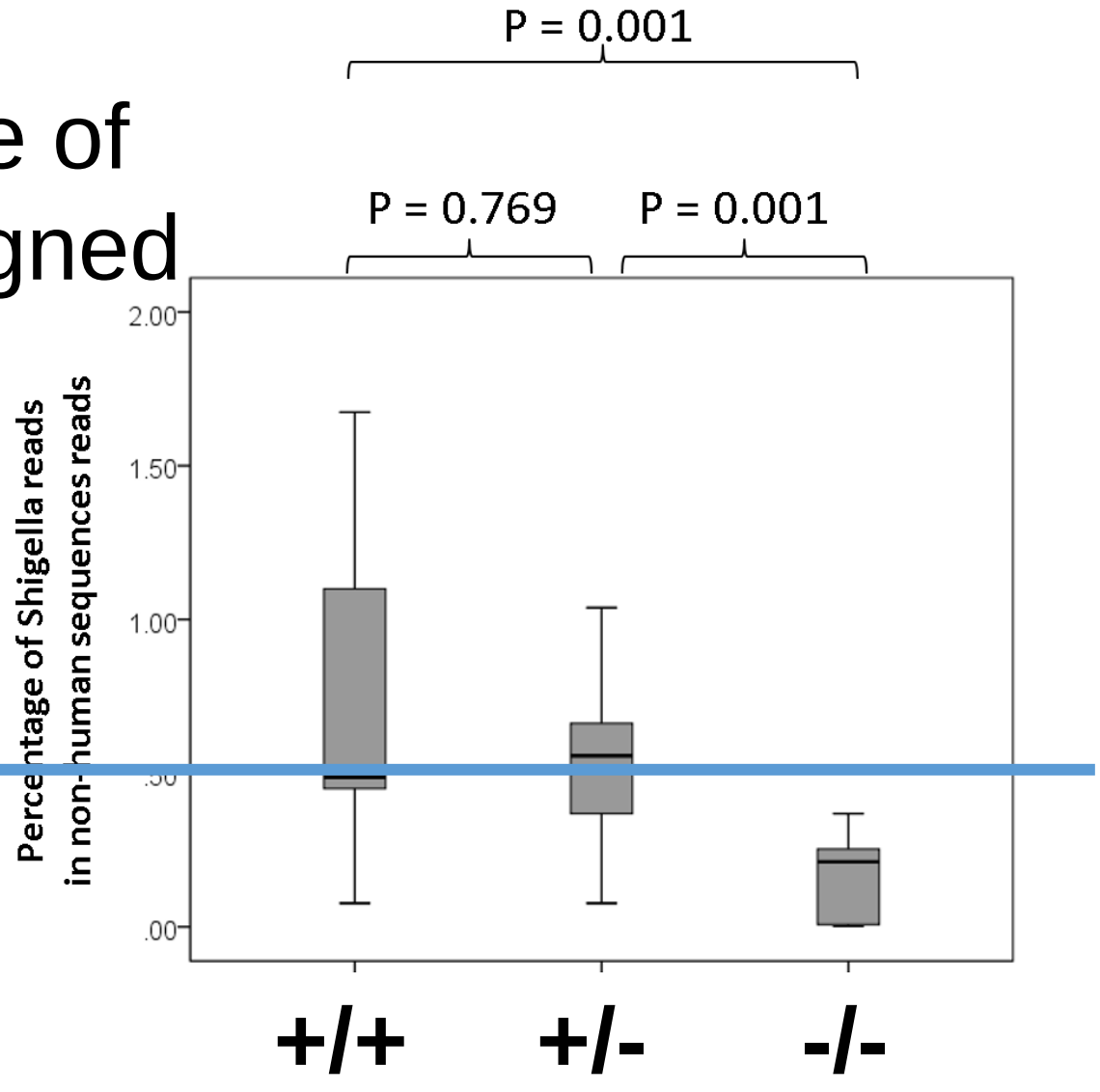


Problem: Only use a small fraction of the data

Count genes assigned to *Shigella* by Kraken

QPCR+Culture- more like QPCR+culture+

Percentage of reads assigned to *Shigella*



Problem: 99.5% are not *Shigella*

0.5%

Four Ways to Count during CHI

All are accurate

Quantitative culture >>> expensive, labor intensive

QPCR >>> specific for target

16S rDNA sequencing >>> detects all taxa in sample

Metagenomic analysis >>> expensive, uses a fraction of the data

Thresholds required for disease causality

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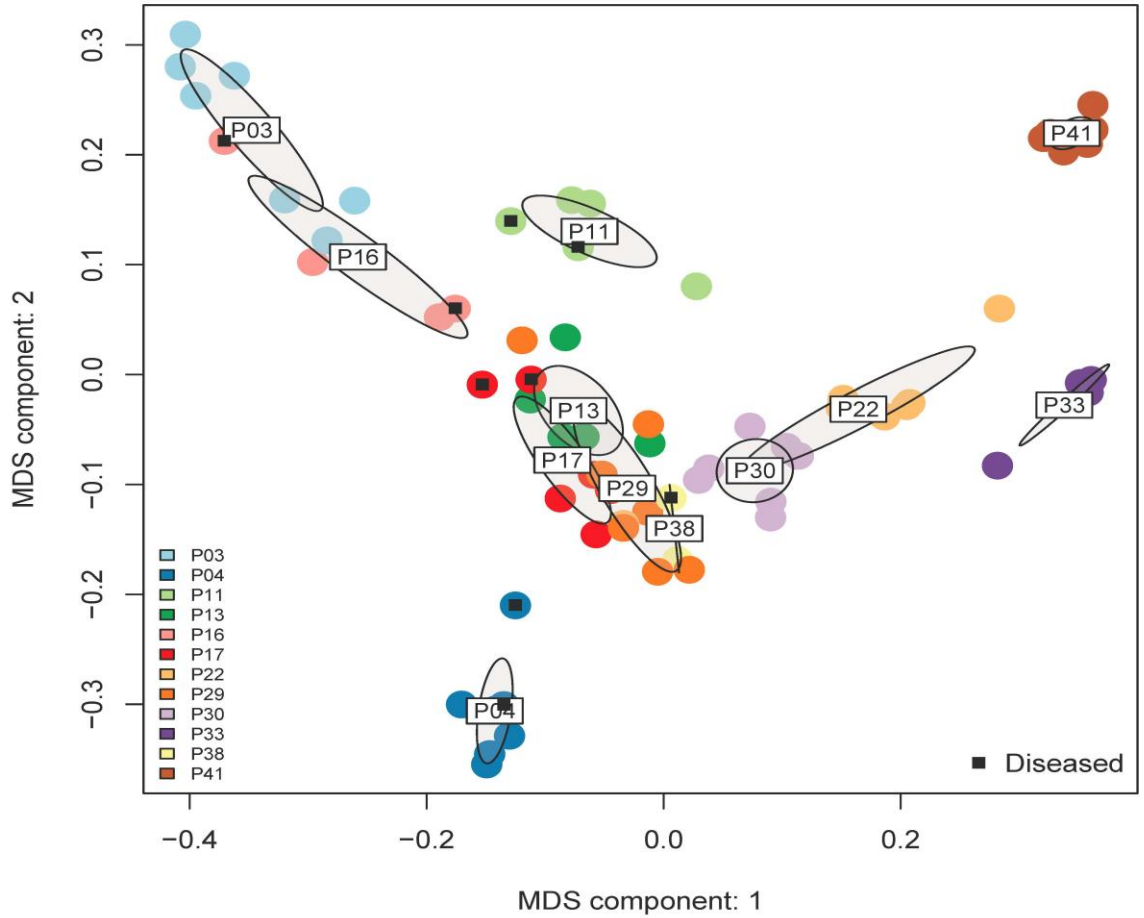
Inter- & Intra- Patient Diversity Before & During Diarrhea

Inter-patient Bray Curtis distances were significantly larger than intra-patient distances.

Diarrheal episodes generally did not induce large shifts (points with black squares).



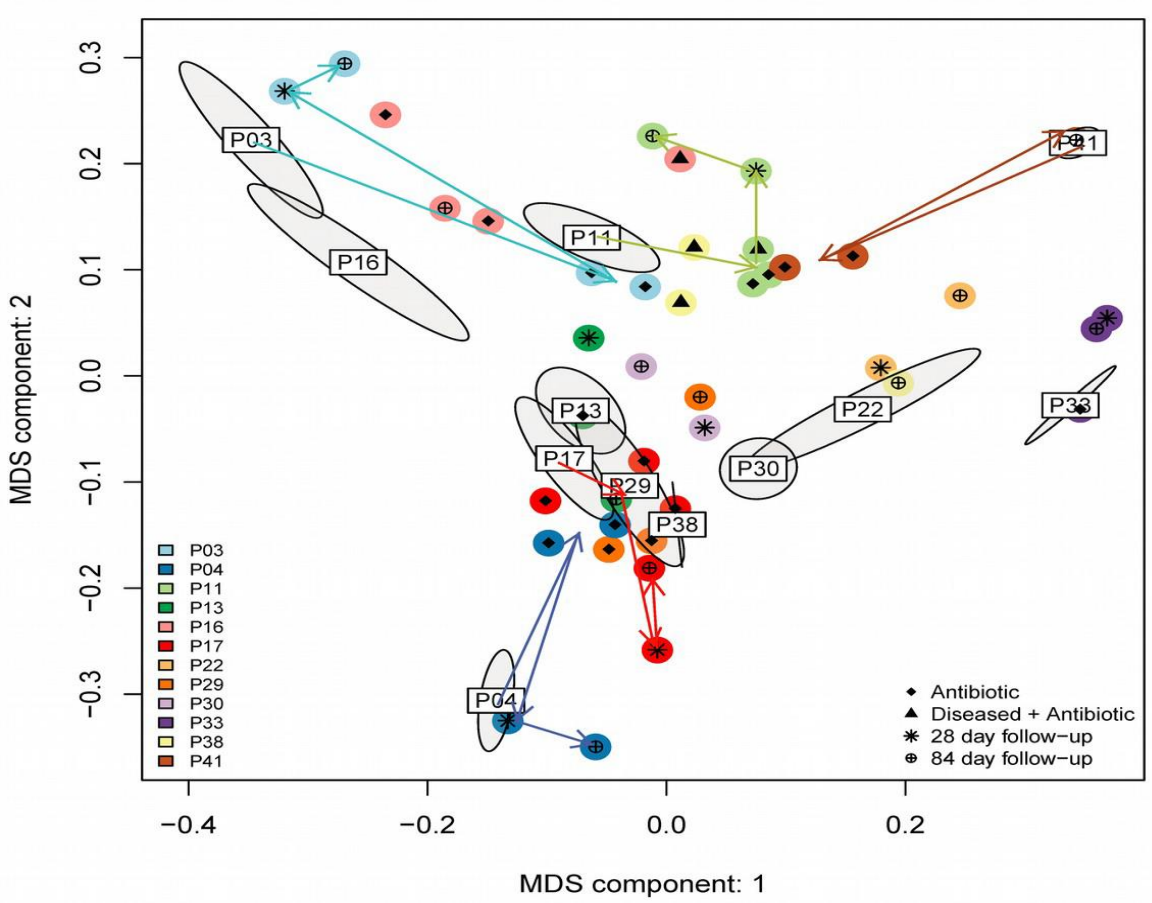
Samples pre-ciprofloxacin treatment

Inter- & Intra- Patient Diversity During & After Ciprofloxacin

Antibiotic treatment induced individual specific large shifts in the microbiota.

Changes were not consistent

Tendency to return to pre-treatment state at 28 and 84 day follow-ups (arrows)



Samples post-ciprofloxacin treatment