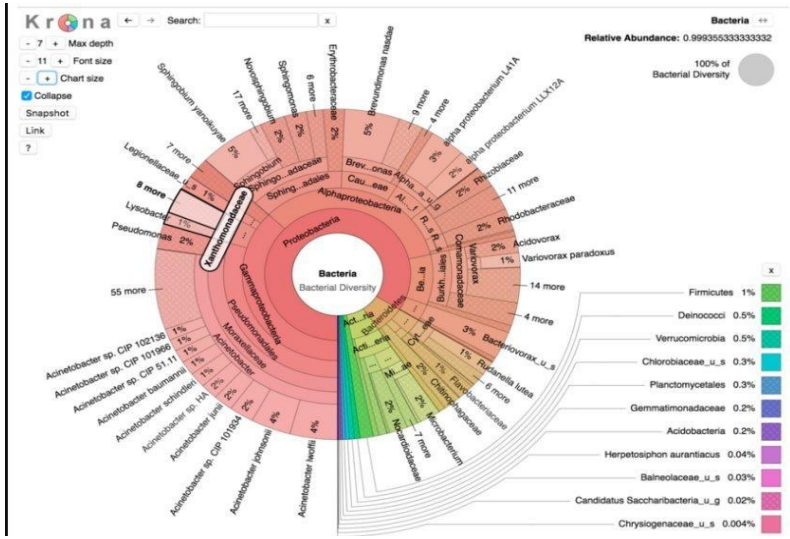
Impact of Antibiotic Therapy on Gut Microbiome and Antimicrobial Resistance in Enteric Fever Patients enrolled in Randomized Controlled Trial

Narayan Kunwar

OUCRU Nepal

Metagenomics: Seeing the Unseen Microbial world

Vs



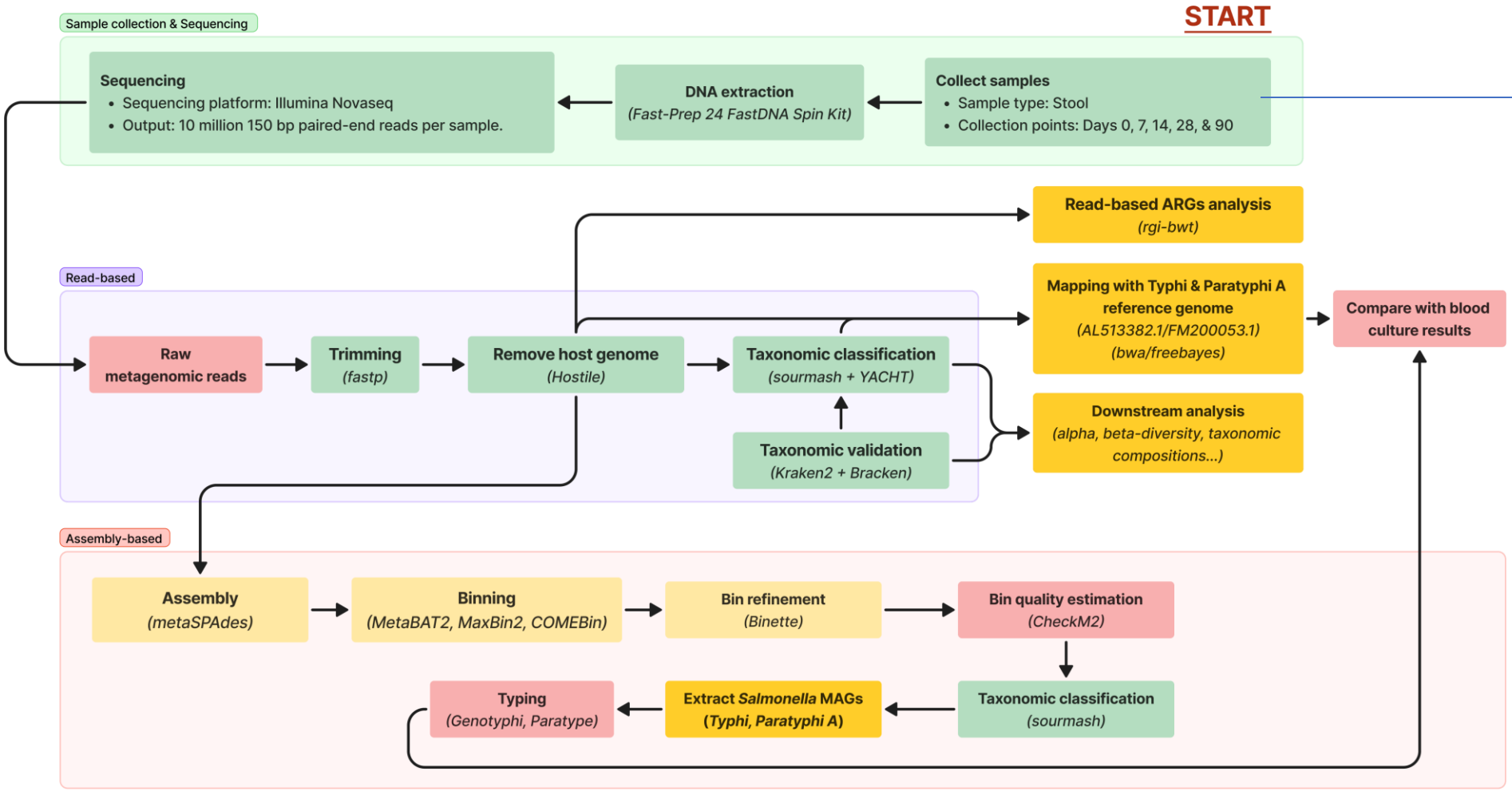
The tiny fraction we can grow

The entire microbial world revealed

Key Research Questions

1. What is the composition and function of the gut microbiota in patients with enteric fever?
2. How do different antibiotic regimens impact the longitudinal changes of antimicrobial-resistant bacteria and the gut microbiome?
3. Can we effectively detect typhoidal Salmonella in the gut microbiota of enteric fever patients?

Methodology



Total: 59 participant samples (51 D0 blood-culture positive, 8 D0 blood-culture negative) and 287 stool DNA samples sent.

Adjusted Timepoint Distribution

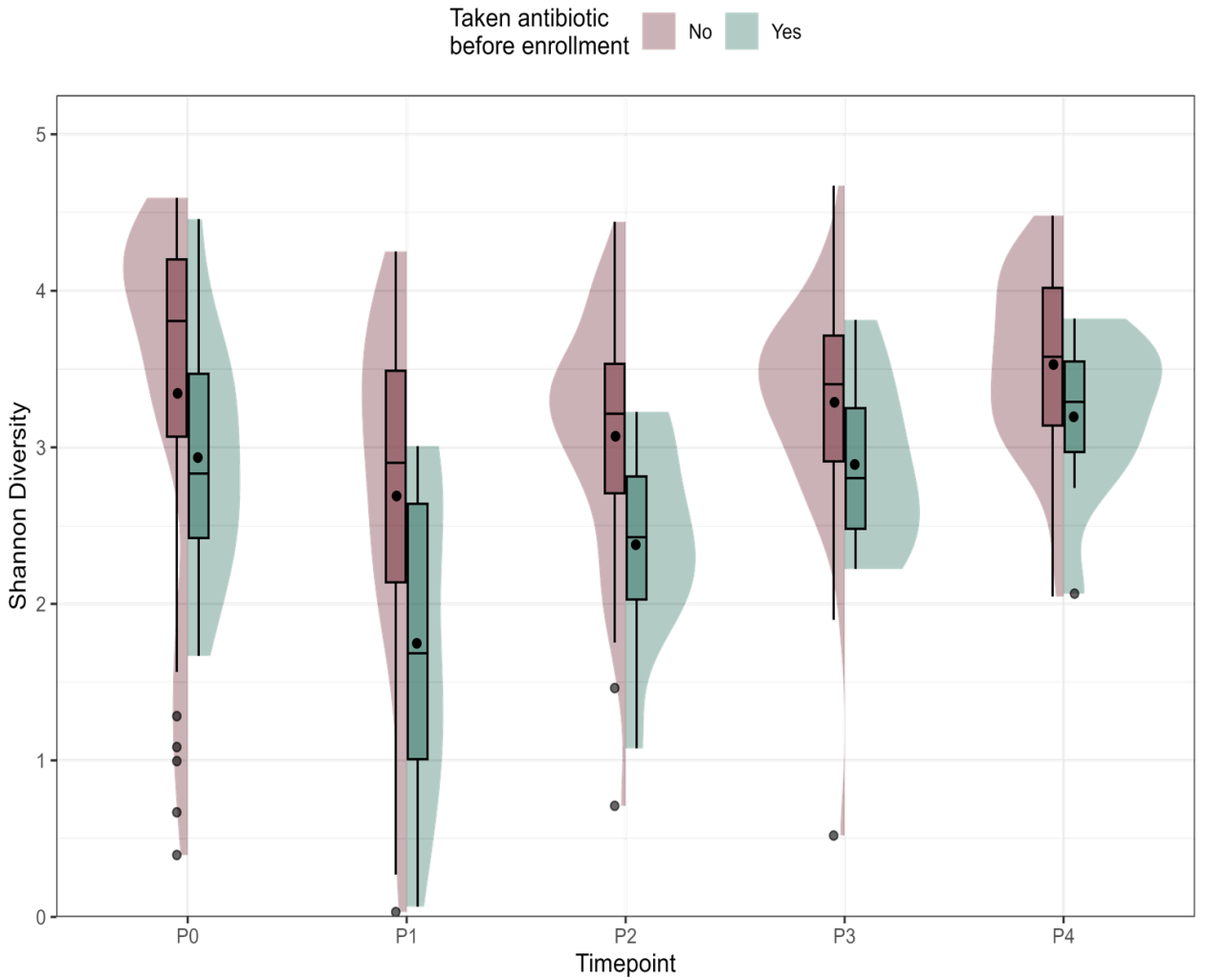
- Selected range (based on collection date)
  - P0: [0;4] (n=52)
  - P1: [5;9] (n=54)
  - P2: [10;19] (n=51)
  - P3: [20;36] (n=55)
  - P4: otherwise (n=55)

Can Typhoidal *Salmonella* Be Detected in the Gut Microbiota of Enteric Fever Patients?

BC result	kraken2_detection	yacht_detection
NG	D20 (*S.enterica* - 0.03024)	
ST	D0 (*S. enterica* - 0.02099)	D0 (SPA - 0.007)
ST	D0 (*S. enterica* - 0.09749)	D0 (ST - 0.11)
SPA	D0 (*S. enterica* - 0.00101)	
ST		D1 (ST - 0.003)
ST		D1 (ST - 0.0008)
ST		D1 (ST- 0.002)
SPA	D0 (*S. enterica* - 0.02209)	D0 (SPA - 0.018); D8 (ST- 0.001)
ST	D15 (*S. enterica* - 0.00194)	
NG	D14 (*S. enterica* - 0.01721)	
ST	D1 (*S. enterica* - 0.00948)	D1 (ST - 0.007)
ST	D0 (*S. enterica* - 0.00231)	
ST		D1 (ST- 0.0005)
ST	D1 (*S. enterica* - 0.00622)	
ST	D0 (*S. enterica* - 0.03988)	D0 (ST - 0.03)

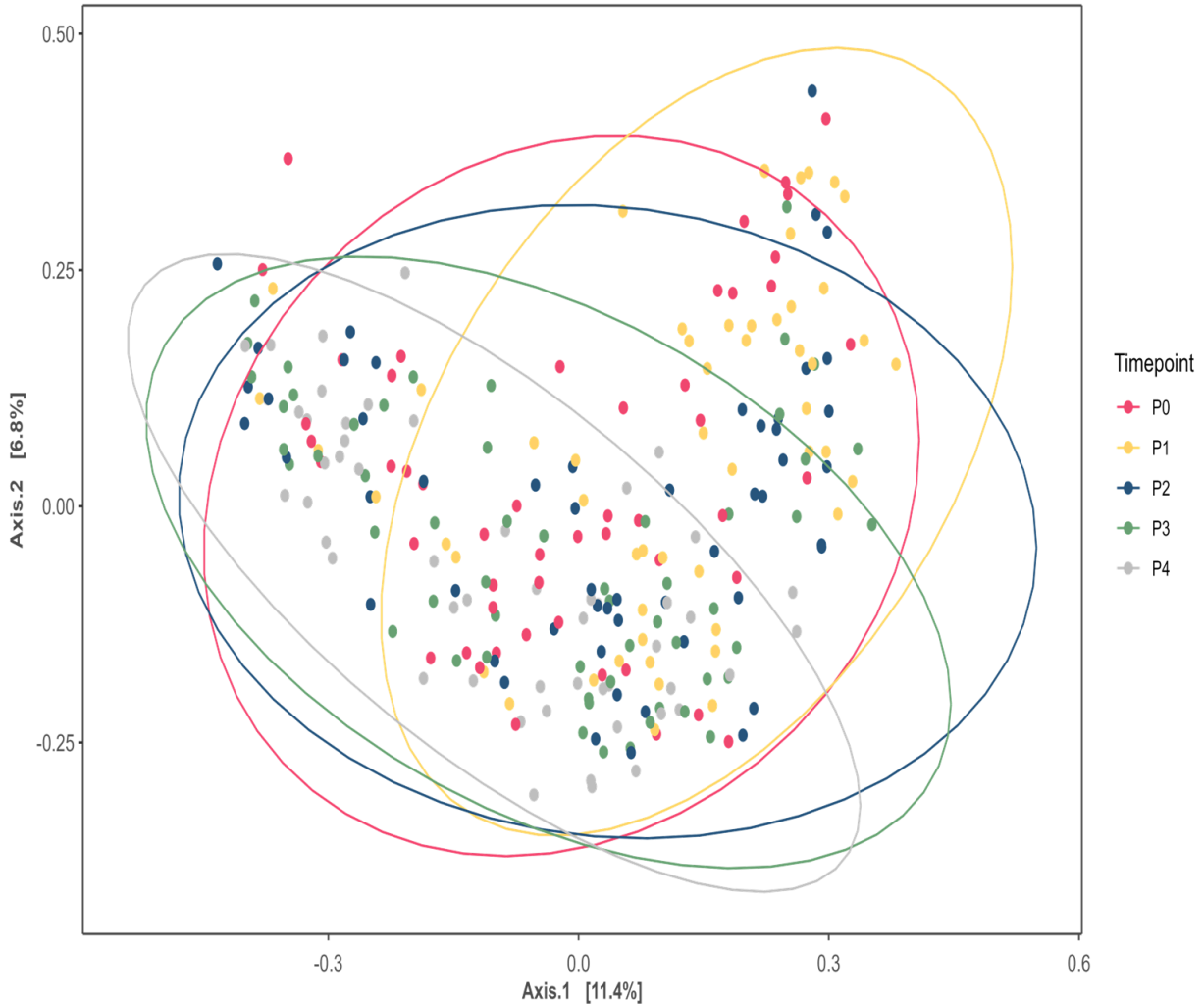
α-diversity (Shannon Index)

- Gut microbiome diversity dropped sharply at P1 compared to baseline (P0) and endpoint (P4).
- By P4, diversity recovered to baseline.
- No significant difference between prior antibiotic users (green) and non-users (pink).
- Both groups showed the same pattern of loss and recovery.



β-diversity (PCoA)

- The P1 group (yellow) shows a clear change in microbial community compared to baseline (pink).
- This shift is seen in both weighted Unifrac and Bray-Curtis analyses.
- P2–P4 move back toward baseline, indicating restoration of the original microbiome composition.
- Both children and adults show the same shift-and-recovery pattern; age does not influence the response.

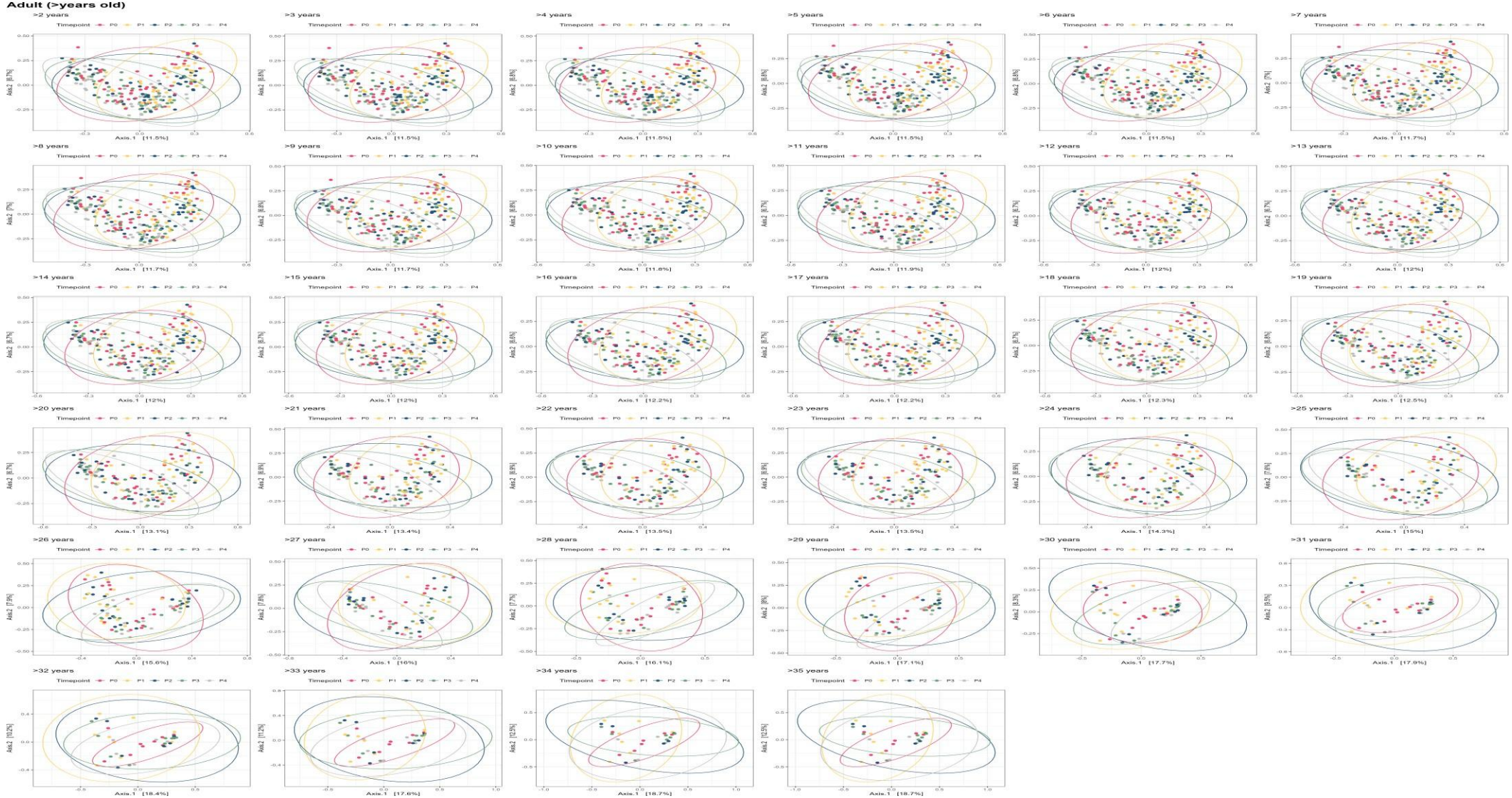


oucru

wellcome

UNIVERSITY OF OXFORD

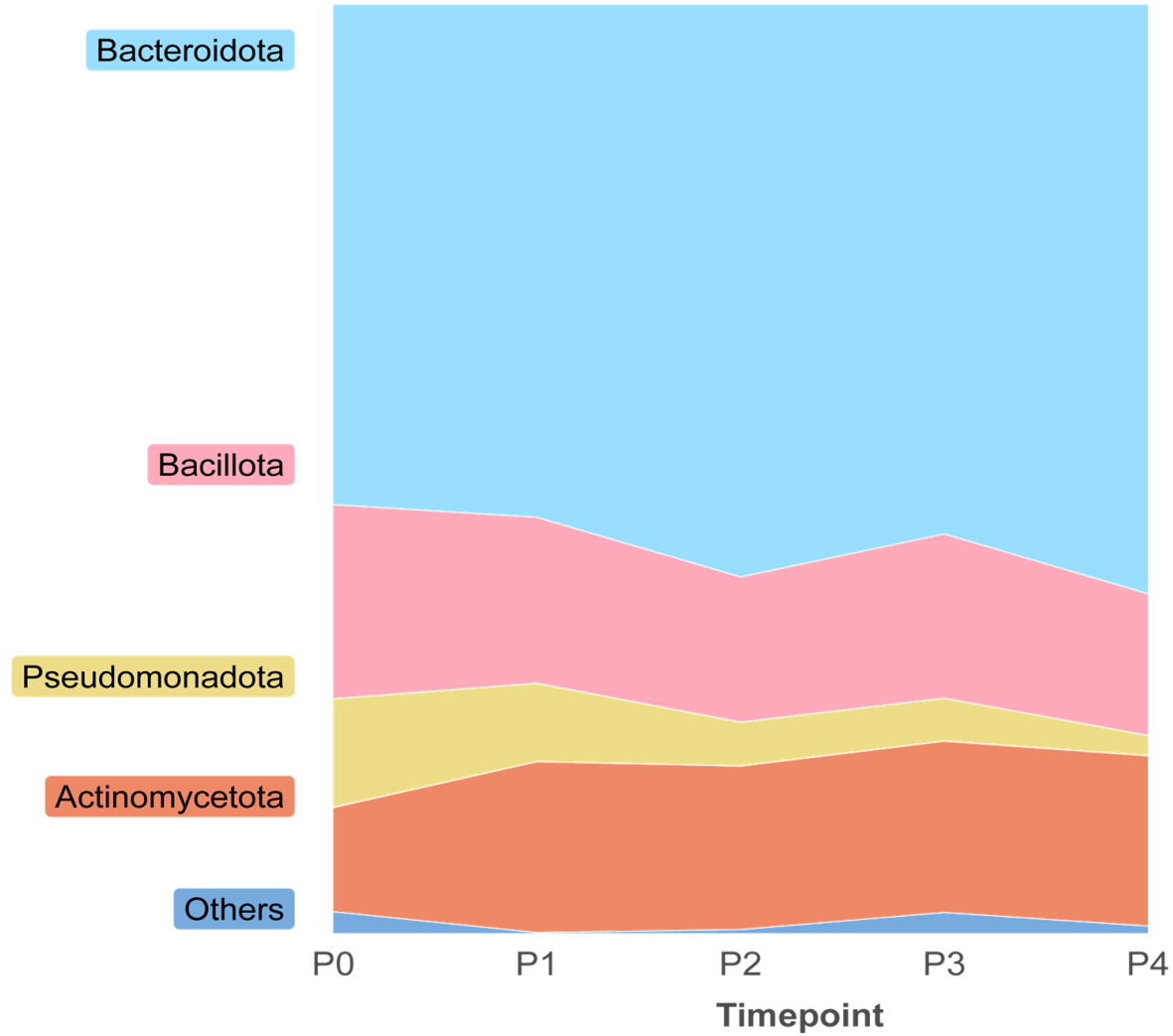
Adult (>years old)



Broad Overview of Gut Bacteria

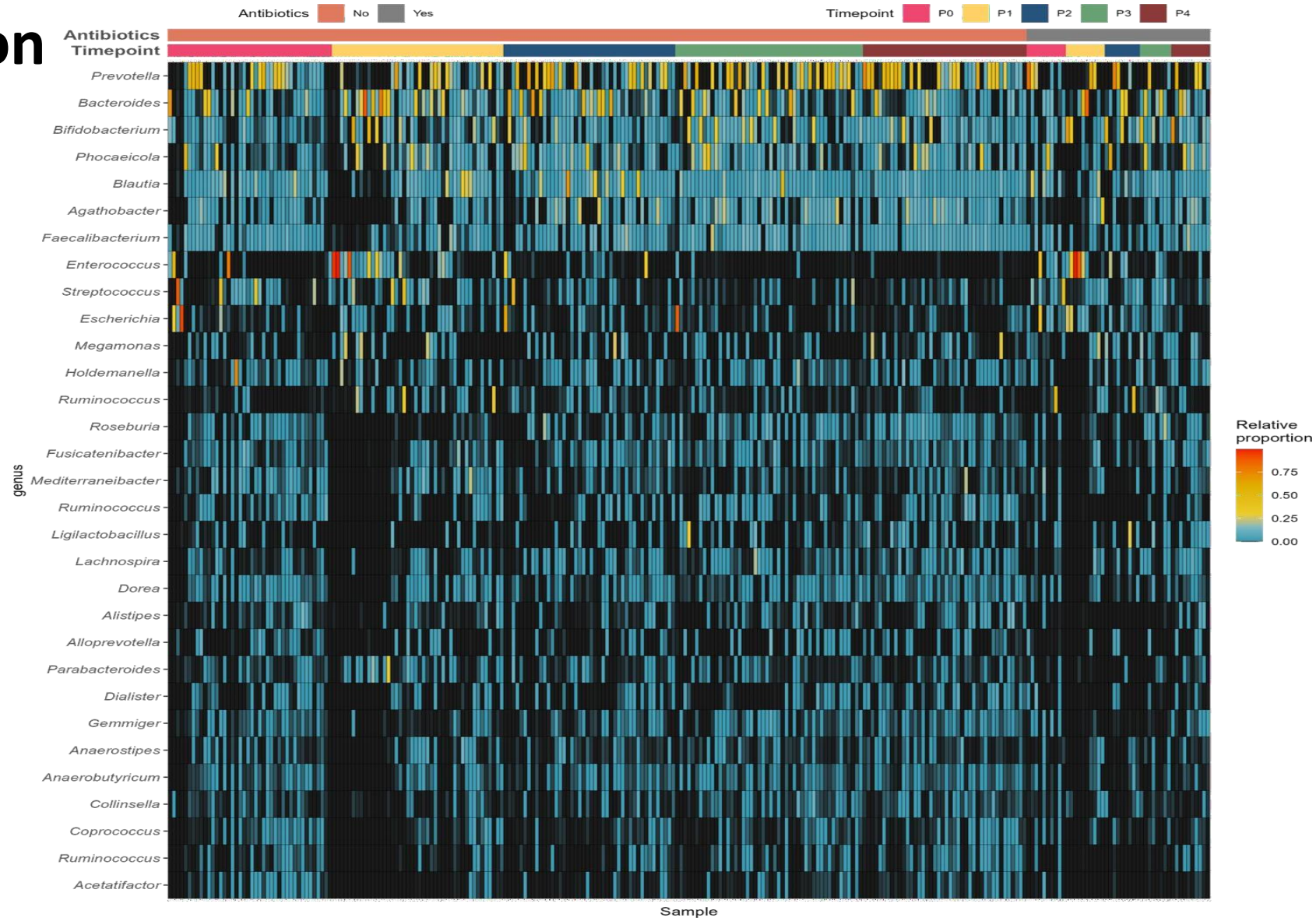
- Gut is dominated by Bacteroidota (blue), common in this population.

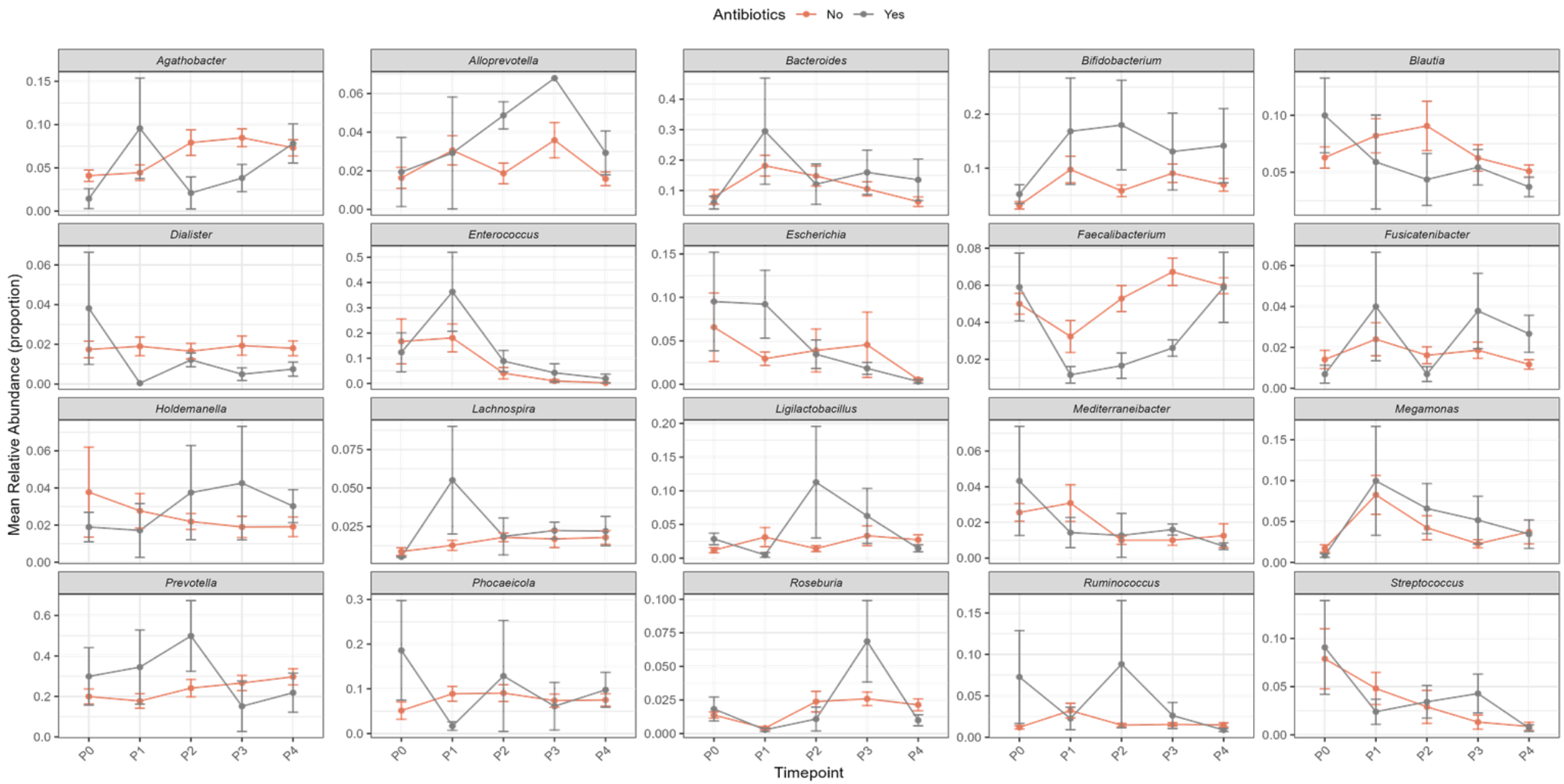
- Bacillota (pink) is the next most prevalent group.



Taxonomic composition (Genus level)

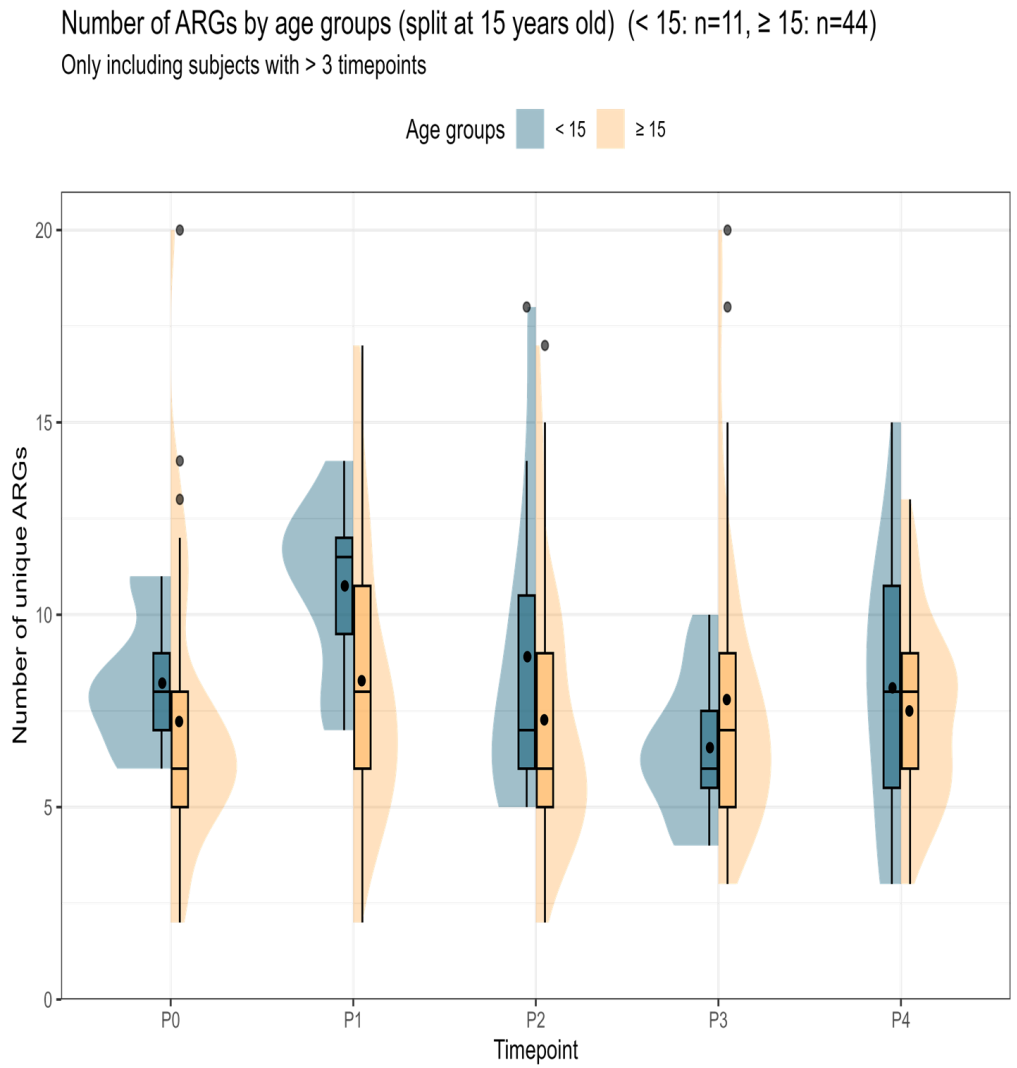
- Most abundant bacteria is *Prevotella*, specifically *Prevotella copri*.
- Darker colors at P1 indicate a sharp decrease in bacterial levels.
- P2–P4 show brightening colors, reflecting bacterial regrowth over time.




Antibiotics
No
Yes
Agathobacter
Alloprevotella
Bacteroides
Bifidobacterium
Blautia
Dialister
Enterococcus
Escherichia
Faecalibacterium
Fusicatenibacter
Holdemanella
Lachnospira
Ligilactobacillus
Mediterraneibacter
Megamonas
Prevotella
Phocaeicola
Roseburia
Ruminococcus
Streptococcus
Mean Relative Abundance (proportion)
Timepoint
P0
P1
P2
P3
P4

Read-based Antibiotic Resistance Genes (ARGs) analysis

- Transient rise in ARGs, peaking at Day 7 (P1).
- Children (<15 yrs): clear spike in ARG levels.
- Adults (≥15 yrs): ARG levels mostly stable.
- Key ARGs: β-lactamases (CfxA, CblA, TEM, CTX-M).
- Notable genes: blaNDM, blaOXA (some emerging by Day 14).
- Overall: Antibiotic pressure briefly expands the resistome, stronger in children

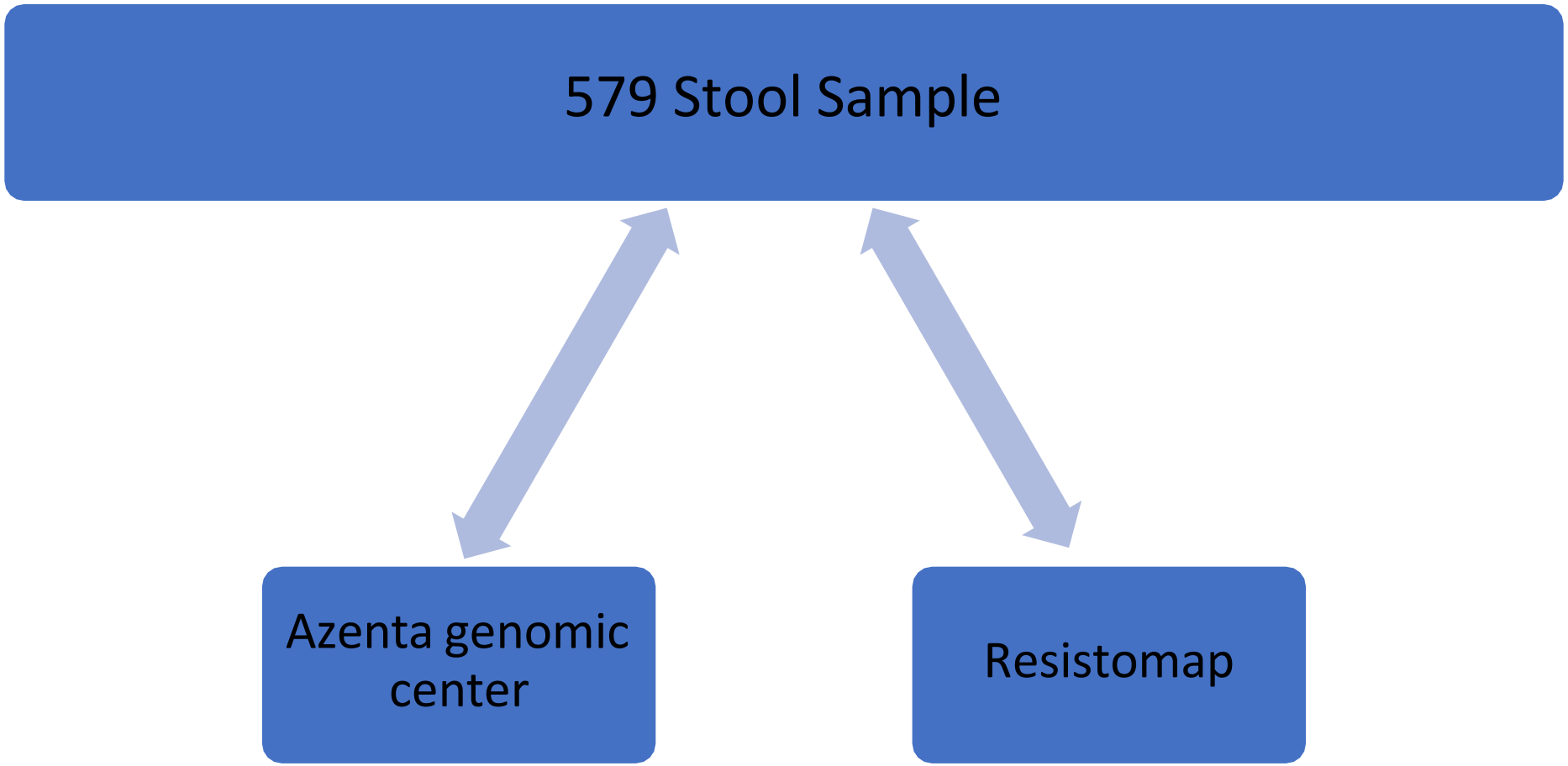


AMR Gene Family	Number of patients	Number of samples
CfxA beta-lactamase	53	135
CblA beta-lactamase	38	72
TEM beta-lactamase	36	58
ACI beta-lactamase	30	81
CTX-M beta-lactamase	19	24
CMY beta-lactamase	18	26
DHA beta-lactamase	17	24
CfiA beta-lactamase	14	19
OXA-48-like beta-lactamase	8	10
NDM beta-lactamase	7	13
OXA-1-like beta-lactamase	6	7
OXA-134-like beta-lactamase	1	1
OXA-58-like beta-lactamase	1	1
macrolide phosphotransferase (mph)	1	1
macrolide esterase	23	31
msr-type ABC-F protein	22	30

Conclusion

1. **Disruption & Recovery:** Antibiotics caused a sharp, transient drop in Alpha Diversity at Day 7 (P1). However, the microbiome is resilient, with diversity returning to baseline levels by Day 90 (P4).
2. **Compositional Shift:** "Good" bacteria (*Prevotella*) temporarily decreased at P1, while opportunistic bacteria (*Enterococcus, Escherichia*) bloomed. This reversed during recovery.
3. **Resistance Spike:** A transient surge in Antibiotic Resistance Genes (ARGs) was observed at Day 7, notably stronger in children (<15 years) compared to adults.
4. **Pathogen Detection:** The optimized metagenomic workflow (Yacht) successfully detected *Salmonella*, effectively distinguishing between infected patients and false positives.

What Next?



Acknowledgements

- Abhilasha Karkey, Sabina Dongol, Chung The Hao, Ton Ngoc Minh Quan, Christopher Parry, Pham Thanh Duy, Buddha Basnyat

Thank you and Speaker Bio

Narayan Kunwar is a microbiologist at the Oxford University Clinical Research Unit (OUCRU) Nepal, specializing in infectious diseases and antimicrobial resistance. His research focuses on generating high-quality evidence to inform clinical practice and public health policy in low-resource settings. He is actively involved in major regional studies, including the ACT–South Asia Trial and the ACORN and ACORN-HAI surveillance platforms. His work integrates microbiology, genomics, and data-driven approaches to better understand and combat emerging infectious threats.