

Child Intestinal Resistome Outcomes of a Randomized Controlled Trial Providing Multiple Micronutrient-Fortified Bouillon Cubes to Households in Northern Ghana

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BACKGROUND

- Large-scale fortification of staple foods and condiments improves dietary micronutrient adequacy and mitigates adverse effects of micronutrient deficiencies.
- However, iron-containing fortified foods or supplements have been associated with gastrointestinal morbidity, including perturbations to the intestinal microbiome.
- Antimicrobial resistance genes (ARGs) are encoded in the gut microbiome and may be implicated in the relationship between unabsorbed iron from micronutrient interventions and disruptions to the intestinal microbiome.
- The extent to which iron-containing fortified foods or supplements influences ARGs in pediatric populations is uncertain.

OBJECTIVE

To evaluate the effect of household use of multiple micronutrient-fortified bouillon cubes on the intestinal resistomes of Ghanaian children aged 2-5 years.

METHODOLOGY

Figure 1. Design of the parent COMIT trial and child microbiome sub-study.

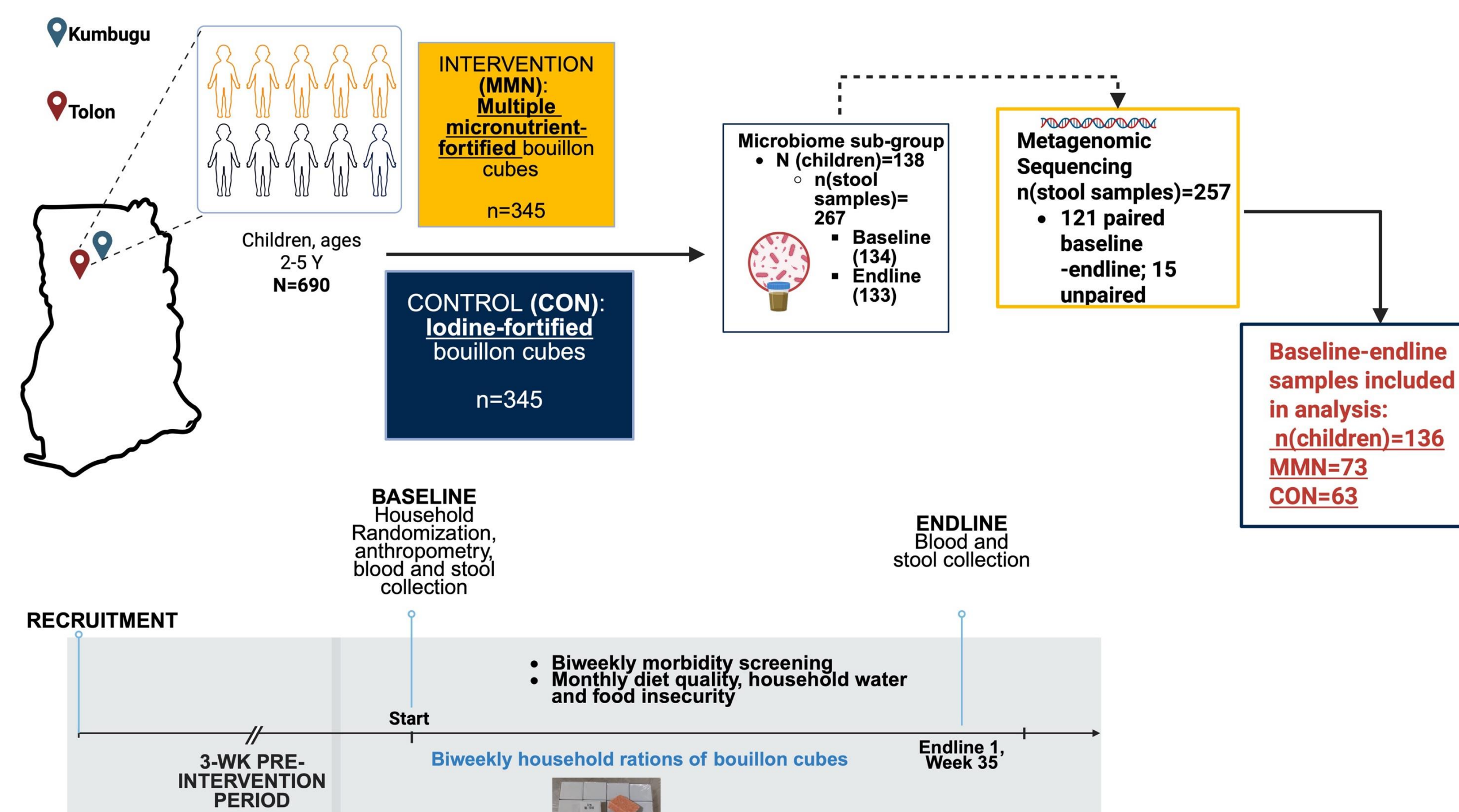


Table 1. Micronutrient composition of study bouillon cubes

Micronutrient	Concentration in MMN fortified bouillon	Modeled % RDA provided for children, 2-5 y*
Vitamin A (µg/g)	200	50–67%
Folic Acid (µg/g)	80	67–89%
Vitamin B12 (µg/g)	1.2	100–133%
Iron (FePP/CA/TSC) (mg/g)	4	40–57%
Zinc (mg/g)	3	60–100%
Iodine (µg/g)†	30	33%

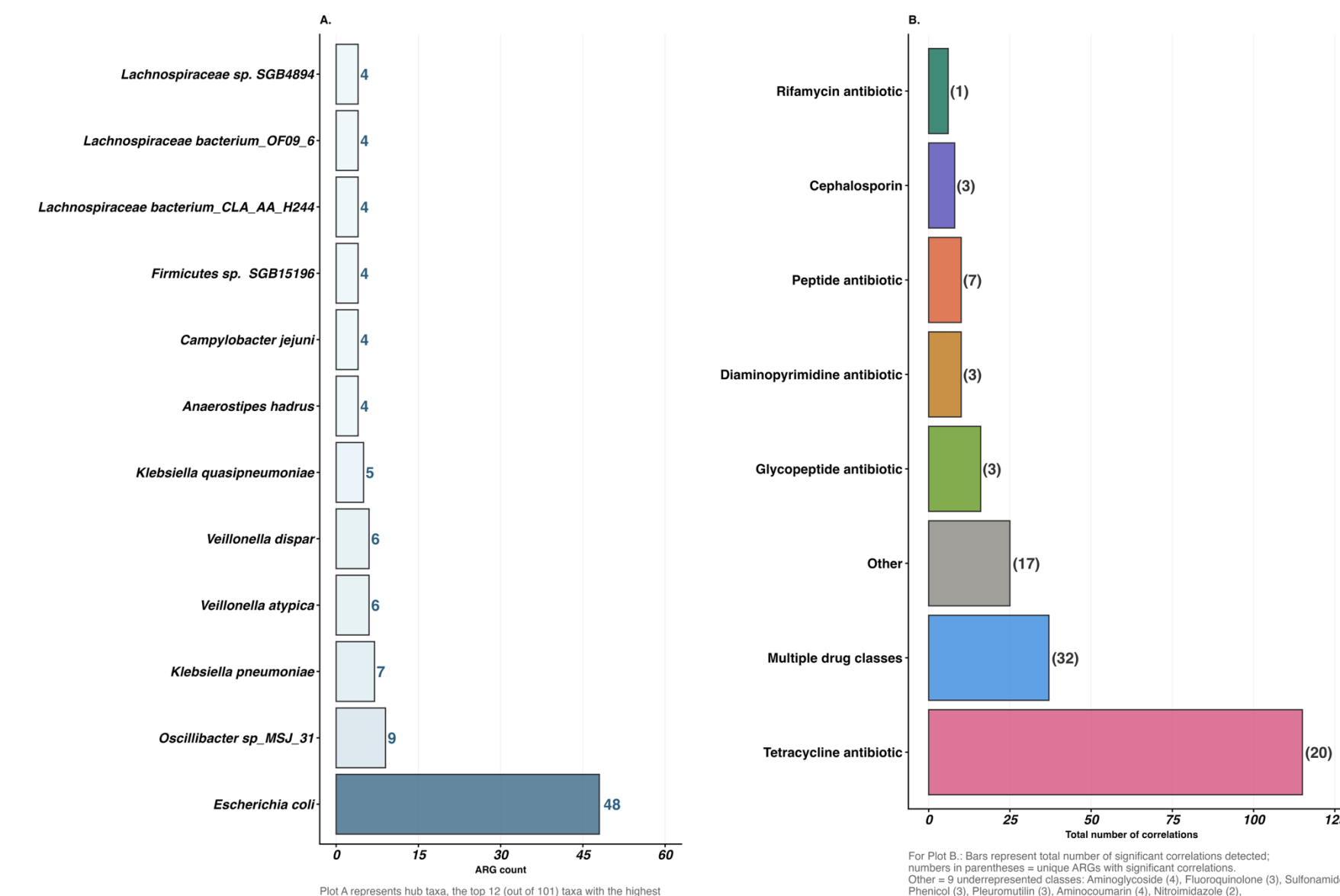
*Assuming child bouillon intake: ~1 g/d; † Equivalent concentration in control cubes

Bioinformatics and Statistical Analysis

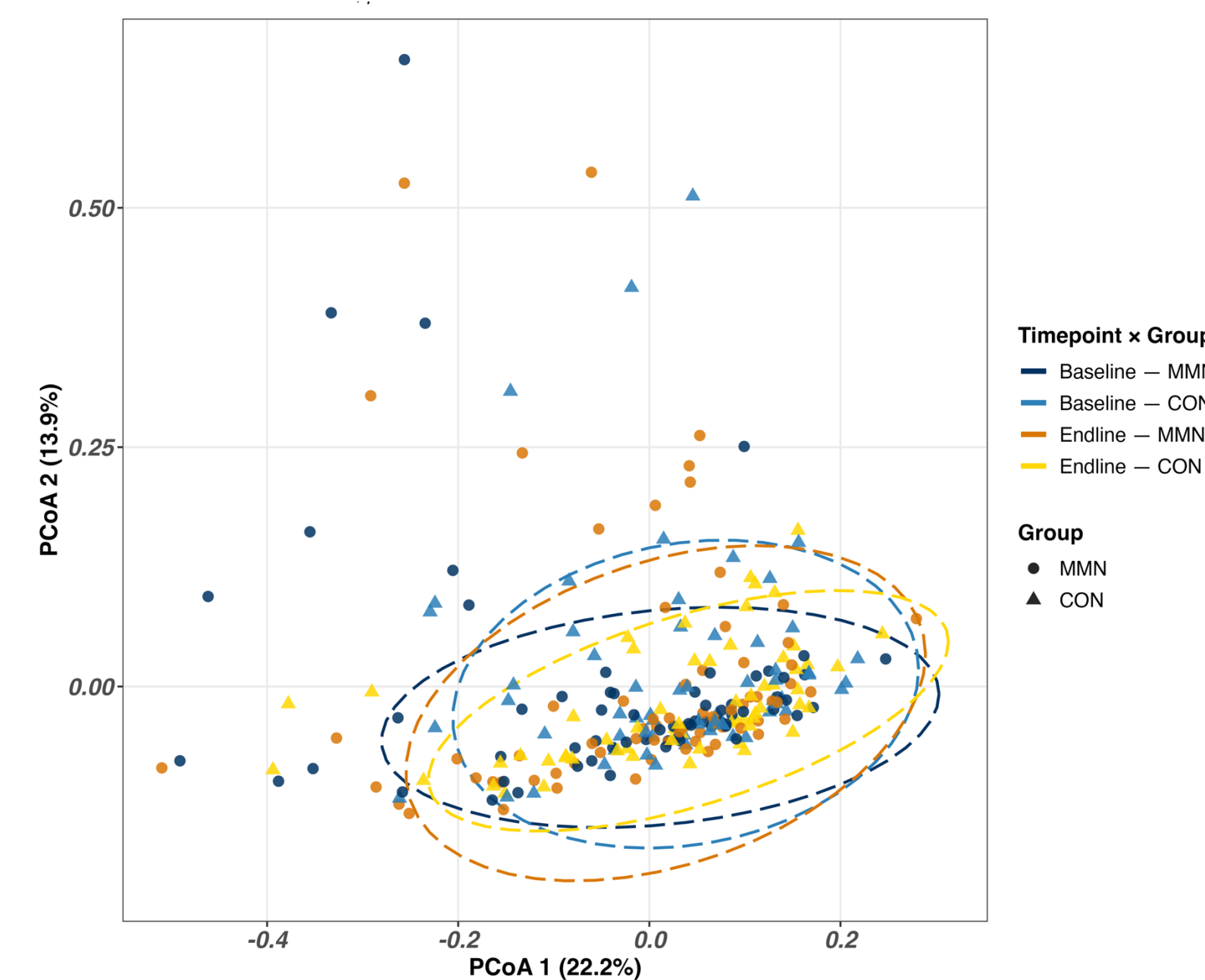
- Resistome Profiling via Comprehensive Antimicrobial Resistance Database, Resistance Gene Identifier.
- Microbial Profiling via MetaPhlan4
- Microbial Taxa-ARG co-occurrence via CCREPE
- Effects on resistome composition via PERMANOVA
- Intervention effects on: ARG abundance (copies per million); ARG richness (absolute gene count) via mixed effects models.
- Gene-level differential abundance via MaAsin3

RESULTS

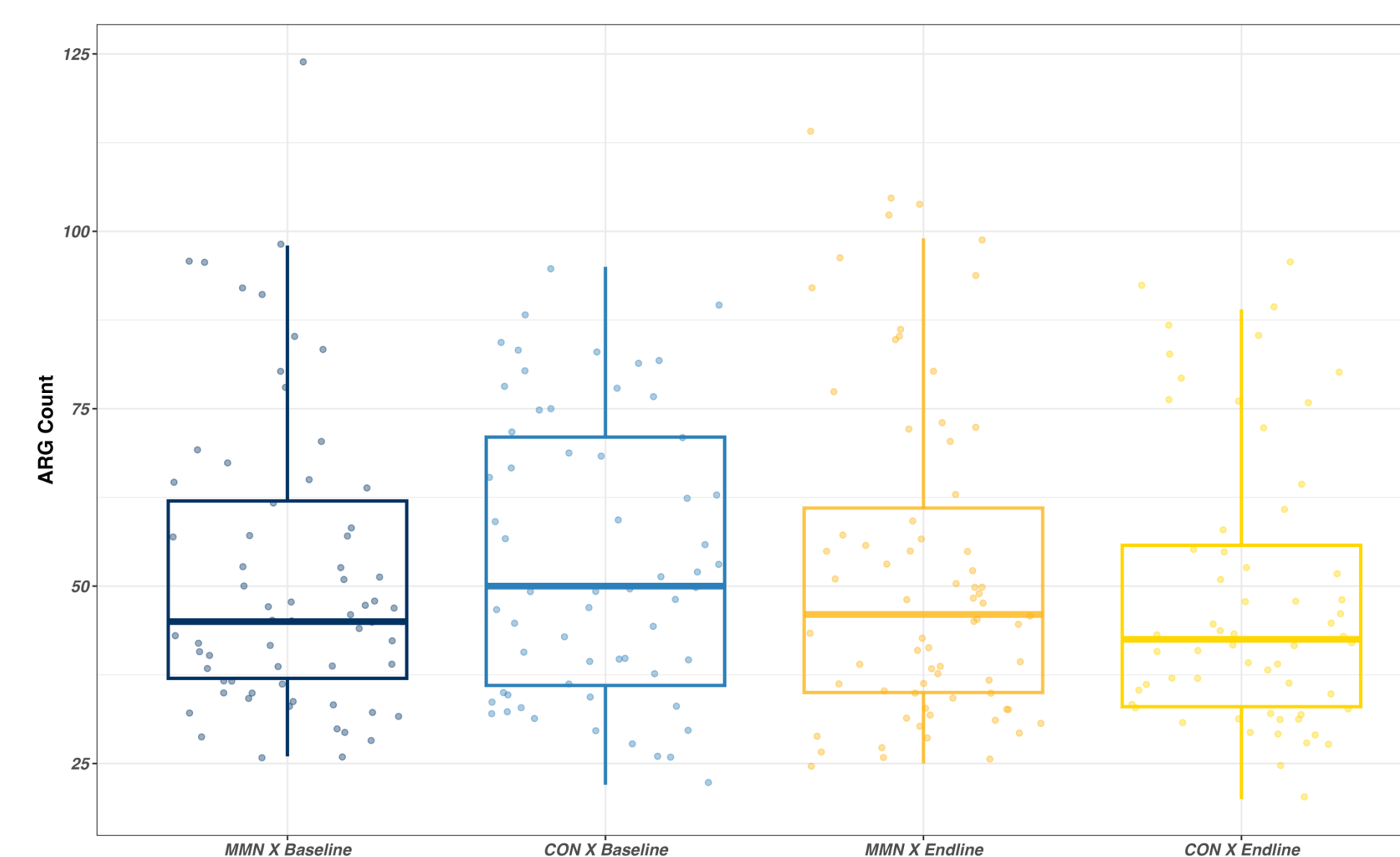
Finding 1. Across all samples, *E. coli* was identified as the species that significantly co-occurred most frequently with ARGs (correlations ≥ 0.3 or ≤ -0.3 with $q < 0.01$) [Panel A]. ARGs conferring resistance to tetracyclines co-occurred with bacterial taxa the most [Panel B].



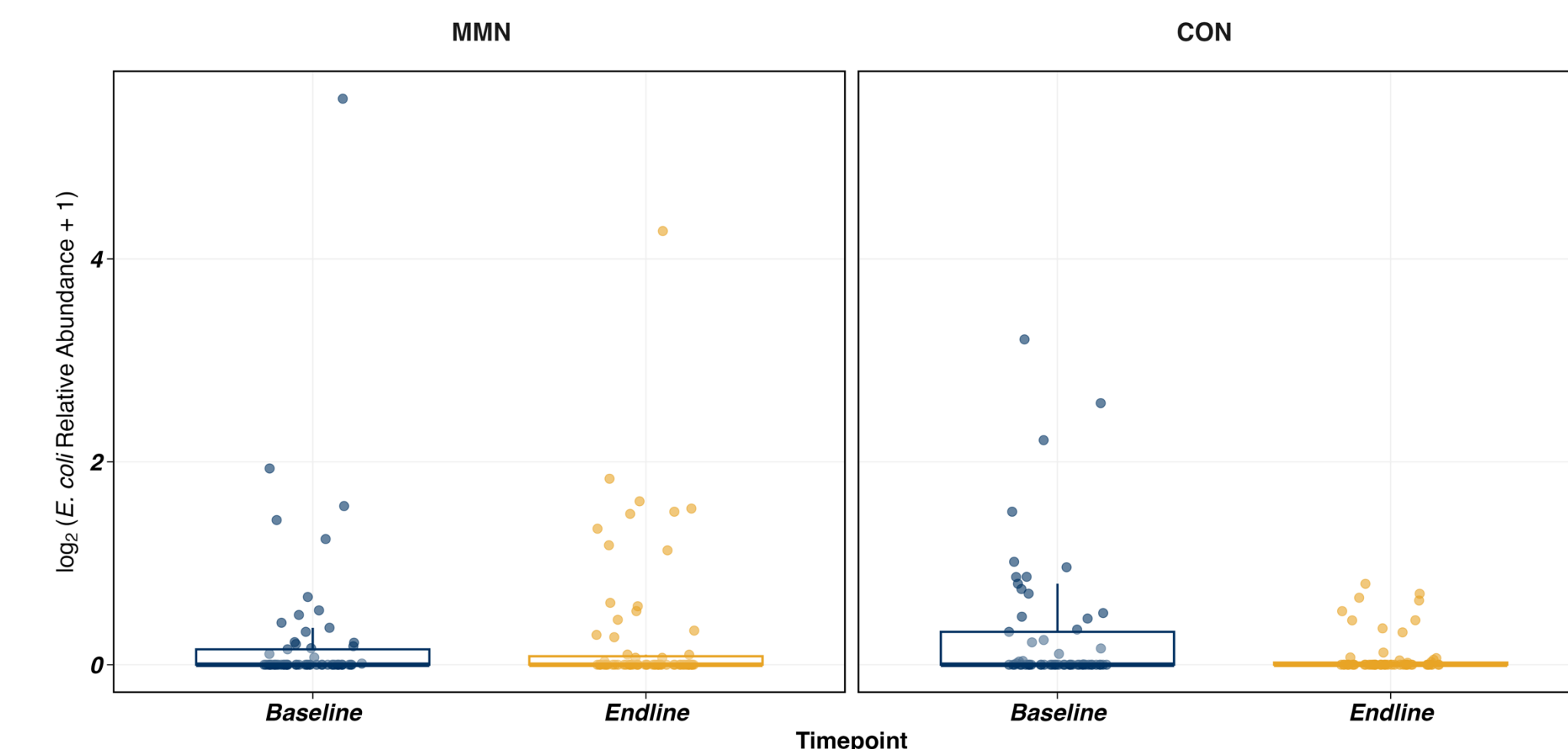
Finding 3. There were no significant changes in the composition of resistomes between groups and over time (PERMANOVA $R^2 = 0.004$; $p = 0.287$)



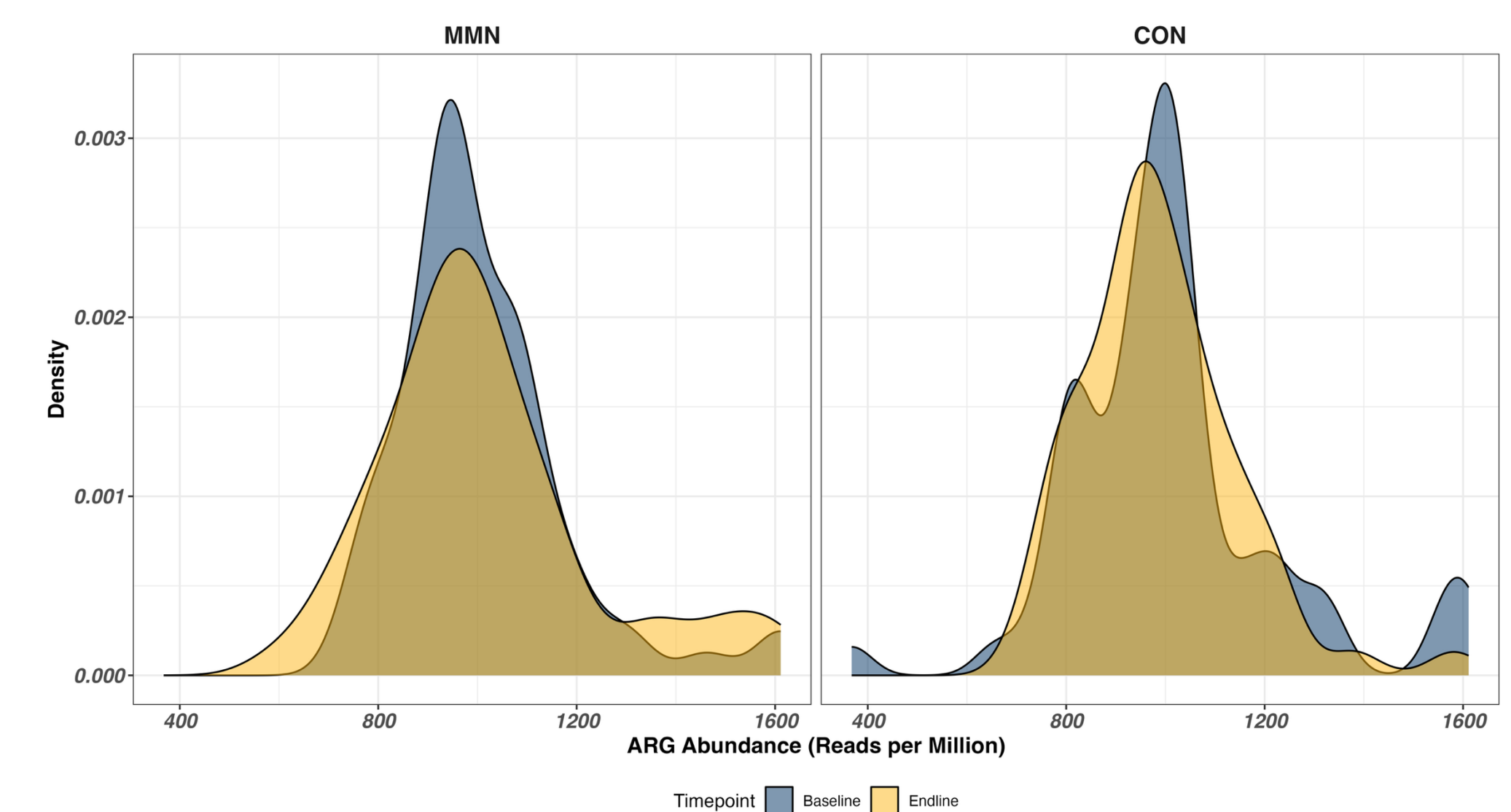
Finding 5. ARG richness remained stable in the MMN group over time whereas the CON group had a 9% decrease (p -interaction, Time x Intervention > 0.001). Median [IQR] Gene count, Baseline vs Endline: MMN (45 [25] vs 46 [26]); CON— 50 [35] vs 43 [23]



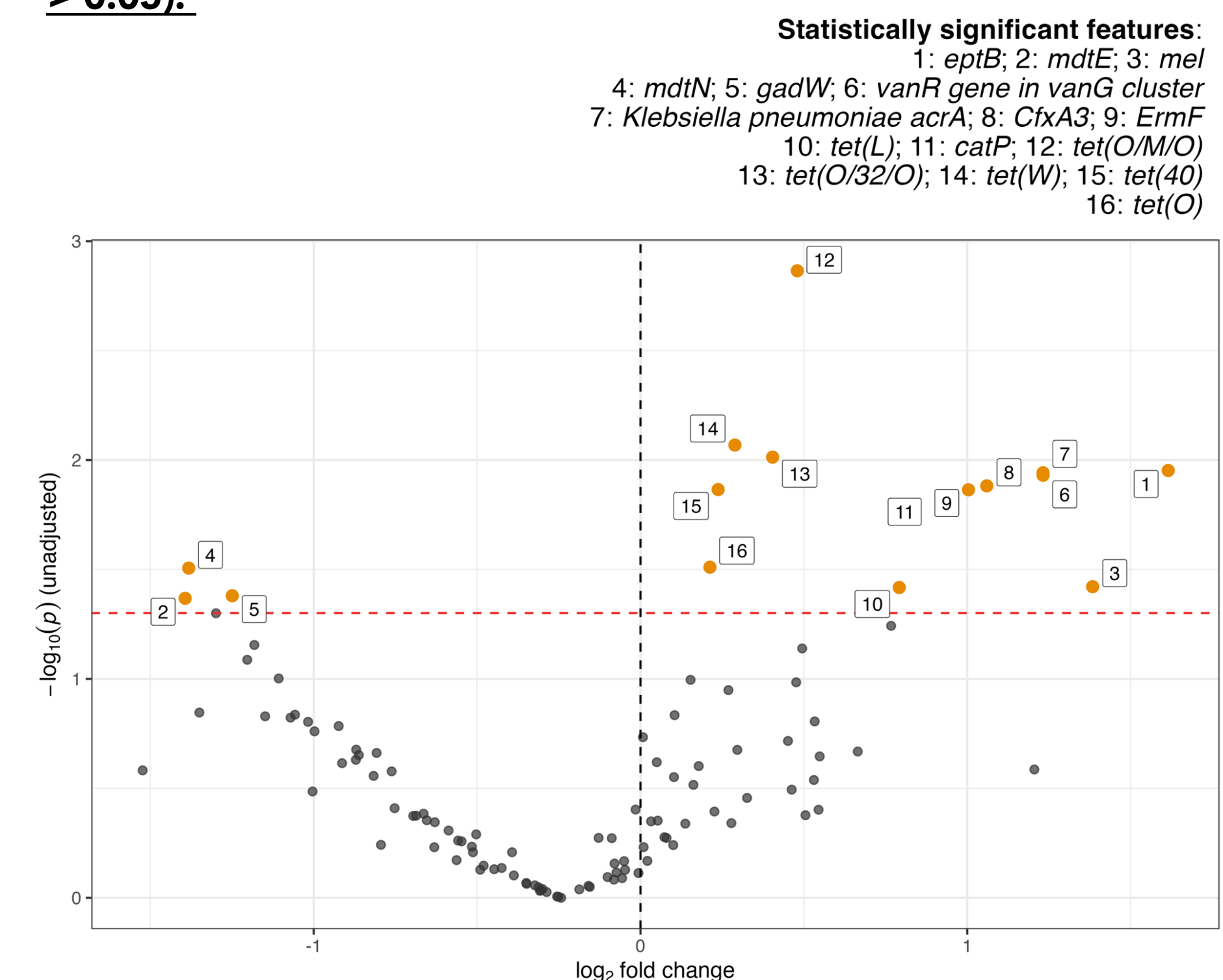
Finding 2. The prevalence and relative abundance of *E. coli* remained low and stable between groups and over time. (p -interaction, Time x Intervention = 0.13; q -interaction = 0.96).



Finding 4. ARG abundance remained stable between groups and over time (p -interaction, Time x Intervention = 0.78). Median [IQR] Reads per Million, Baseline vs Endline: MMN—970.1 [172.1] vs 978.4 [222.7]; CON— 987.9 [139.3] vs 963.3 [171.9]



Finding 6. Sixteen ARGs showed nominal differential abundance (p -interaction, Time x Intervention < 0.05), but none remained significant after adjustments for multiple comparisons (q -interaction > 0.05).



CONCLUSION

Multiple micronutrient-fortified bouillon cubes had no clinically significant effects on gut resistomes of children in this context, suggesting minimal risk of ARG-mediated gastrointestinal or infectious morbidity.



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