

No Evidence of Perturbations of the Breast Milk Microbiome by Antiretroviral Therapy

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BACKGROUND

Antiretroviral treatment (ART) reduces AIDS-related morbidity and mortality in women living with HIV (WLWH) and has broken the chain of mother-to-child transmission (MTCT) of HIV-1 both peripartum and through breastfeeding.

- Breastfeeding has profound benefits including reducing infant mortality.
- Human milk contains over 200 beneficial factors including cells, antibodies, human milk oligosaccharides and beneficial microbes
- The milk microbes contribute to infant gut colonization and the gut microbiome is critical for infant immune development and general health.
- Some data indicate that ART may alter the microbiome of people LWH.
- We investigated whether ART altered the breast milk microbiome.

METHODS

To determine if maternal ART altered the breast milk microbiome, we performed 16S rRNA gene sequencing of the V4 region on breast milk samples from WLWH randomized to receive maternal antiretroviral therapy (mART) with TDF/FTC/LPVr versus no maternal ART (infants received nevirapine for MTCT prophylaxis (Control Group)) for the duration of breastfeeding in a Clinical Trial¹.

- 50 WLWH: 25 on mART; 25 no maternal ART (infants received NVP (iNVP))
- 16SrRNA gene sequencing of the V4 region from breast milk samples at 6, 26, 50 and 74 weeks postpartum
- Participants matched on baseline maternal CD4 count, viral load, country, and date of study randomization.
- DADA2, decontam, and phyloseq were used for sequence inference, contaminant removal, and subsequent analyses.
- Differences in overall microbiome composition were assessed using permutational multivariate ANOVA with Jenson-Shannon distances.
- To account for repeated measures, linear mixed effects models were used to identify specific taxa that differed between the treatment groups.
- All statistical analyses were performed using ‘R’ v4.1.3.

Variable (mean (SD))	Infant Nevirapine	Maternal ART	p-value
N	25	25	
Country			
Malawi	16 (64%)	16 (64%)	
South Africa	7 (28%)	7 (28%)	
Uganda	1 (4%)	1 (4%)	
Zimbabwe	1 (4%)	1 (4%)	
Parity prior to Delivery	1.7 (1.3)	1.6 (1.2)	0.91
Gestational Age at Delivery (wks)	38.5 (3.3)	38.3 (2.2)	0.81
Baseline CD4 Count (cells/mm ³)	555 (166)	557 (166)	
Plasma Log ₁₀ HIV-1 RNA	3.85 (0.97)	3.63 (0.86)	

1. Flynn PM, Taha TE, Cababasay M, Fowler MG, Mofenson LM, Owor M, et al. Prevention of HIV-1 transmission through breastfeeding: Efficacy and safety of maternla antiretroviral therapy versus infant nevirapine prophylaxis for duration of breastfeeding in HIV-1-infected women with high CD4 cell count (IMPAACT PROMISE): a randomized, open label, clinical trial. JAIDS 2018;77:383-392.

The breast milk microbiome is **NOT** perturbed by combination antiretroviral therapy in this study.

RESULTS

- Microbiome profiles were obtained for 196/200 samples following standard processing and filtering criteria from 50 women (25 mART, 25 iNVP).
- Overall, breast milk microbiomes were similar between women receiving ART (mART) and those not on ART (iNVP), with *Streptococcus* and *Staphylococcus* as the dominant members (Figure 1).
- PERMANOVA revealed that time since delivery and the microbiome were associated as expected (R2=0.037, p<0.001), but maternal ART was not a significant driver of overall microbiome variation (Figure 2; R2=0.001, p=0.845).
- No statistically significant differences in diversity at any of the four timepoints nor in genus- or species-level relative abundances between the treatment arms were observed when using a linear mixed-effects model.

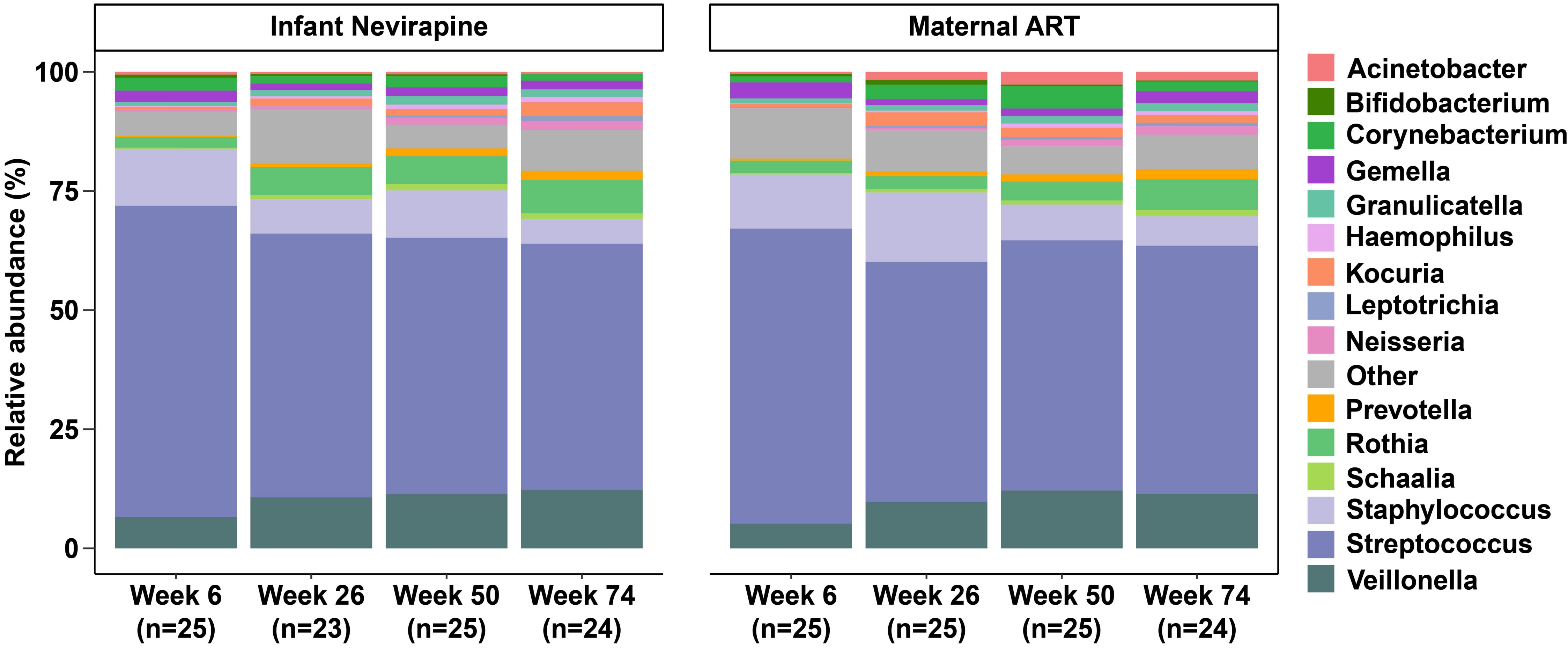


Figure 1: Composite histograms of the breast milk microbiome in women whose infants were receiving nevirapine prophylaxis versus women receiving combination ART show no statistically significant differences in diversity, genus- or species level abundances at any of the 4 timepoints.

CONCLUSIONS

- An important negative study
- We found no evidence that the breast milk microbiome is altered by ART in lactating women living with HIV compared with women LWH and not on ART.
 - This study should alleviate concerns that antiretroviral therapy may interfere with key microbes that are transferred from mom to baby.

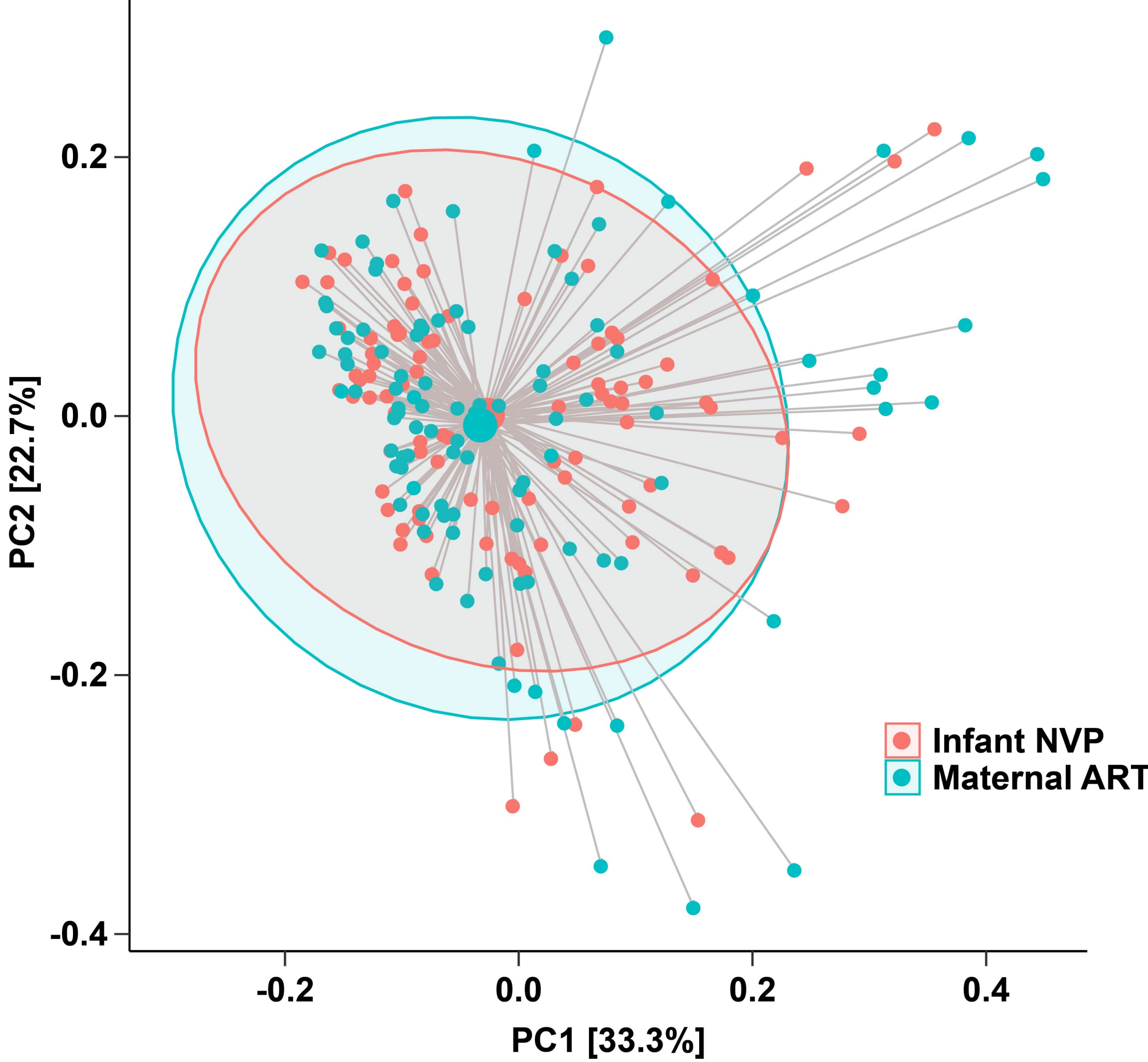


Figure 2: PCoA of infant nevirapine group versus maternal ART demonstrates that maternal ART was not a driver of overall microbiome variation (p=0.845).

FUTURE DIRECTIONS

- Future investigations will evaluate how the breast milk metabolome is altered by antiretroviral therapy

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