

Human Milk Microbiome-Derived Lactotypes are Associated with Growth and Brain Development in Very Preterm Infants

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Introduction

Very preterm infants represent a uniquely vulnerable population due to immature intestinal and immune systems and heightened risk for suboptimal growth and brain development in the NICU.¹⁻³

Human milk is a modifiable influence of these outcomes and contains diverse communities of microorganisms—the human milk microbiome—that may be beneficial for very preterm infants.^{4,5}

Yet few studies have examined the role of human milk microbiota in growth and brain development among very preterm infants.

Objective

To identify human milk lactotypes, or clusters of microbial taxa with similar community structures, and determine associations with growth and brain development in very preterm infants.

Materials and Methods

Prospective cohort of 134 infants <32 weeks' gestation.⁶

Human milk samples collected at 2 weeks' chronological age and 36 weeks' postmenstrual age (PMA); bacterial DNA extracted via PCR; human milk microbiome via 16S rRNA sequencing.⁷

Lactotypes defined using K-means, which clusters samples based on similarities in genus-level microbial composition.

Outcomes at term-corrected age: anthropometric z-scores, body composition z-scores (via air displacement plethysmography); brain volumes (via magnetic resonance imaging and MANTiS).

Linear mixed models to estimate differences in outcomes across lactotypes at 2 weeks' chronological age and 36 weeks' PMA.

Results

Table 1: Participant Characteristics (Mean ± SD or %).

Mothers	
N	123
Age at delivery, years	31.9 ± 5.3
Pre-pregnancy BMI, kg/m ²	27.7 ± 6.9
Very Preterm Infants	
N	134
Gestational age, weeks	28.4 ± 2.0
Female, N (%)	48 (42)
Birthweight, grams	1147 ± 358

Figure 1: Human Milk Lactotypes Differed at 2 Weeks' Chronological Age (A) and 36 Weeks' PMA (B).

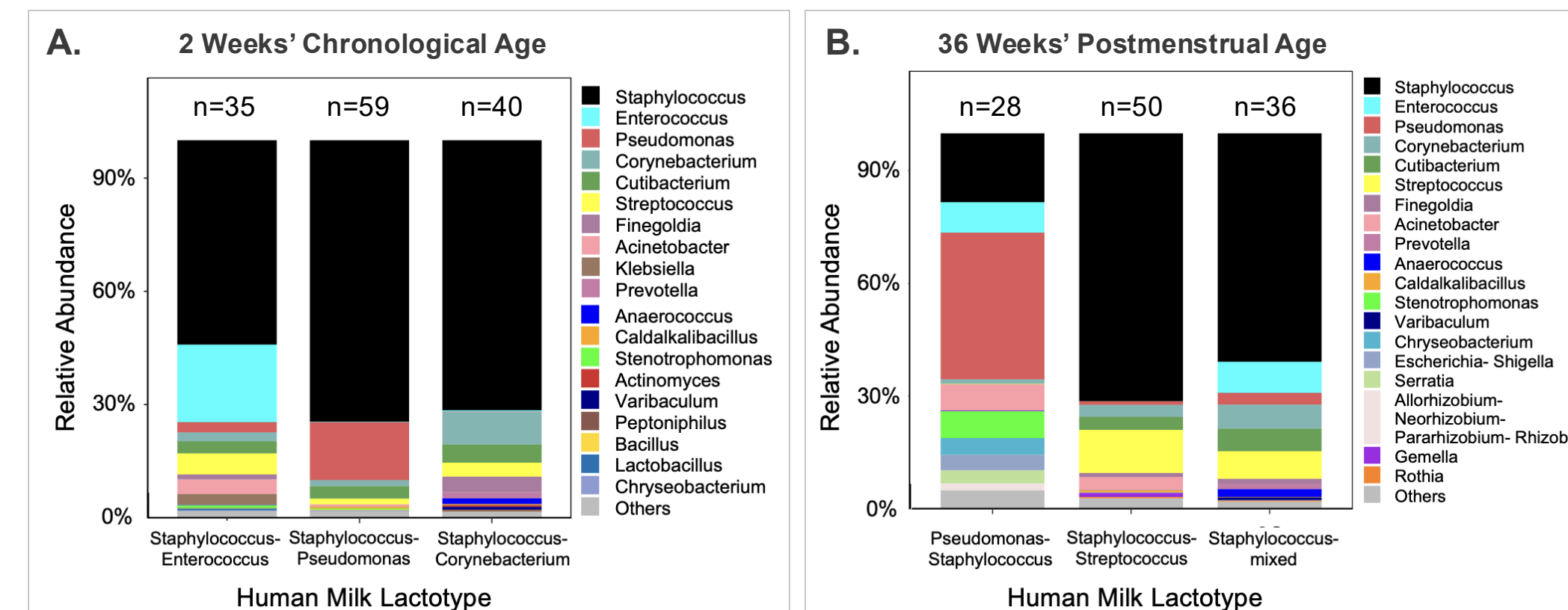


Figure 1A. Human milk lactotypes defined by their dominant bacterial taxa. At 2 weeks' chronological age, three distinct lactotypes were identified as *Staphylococcus-Enterococcus*, *Staphylococcus-Pseudomonas*, and *Staphylococcus-Corynebacterium*.
Figure 1B. Human milk lactotypes defined by their dominant bacterial taxa. At 36 weeks' postmenstrual age, three distinct lactotypes were identified as *Pseudomonas-Staphylococcus*, *Staphylococcus-Streptococcus*, and *Staphylococcus-mixed*.

Table 2: Differences in Growth and Brain Development Outcomes Between Lactotypes at 36 Weeks' PMA.

	Staphylococcus-Streptococcus	Staphylococcus-Mixed		Staphylococcus-Streptococcus	Staphylococcus-Mixed
Weight Z	0.30 (0.01, 0.60)	0.19 (-0.13, 0.51)	Fat Mass Z	0.71 (0.14, 1.29)	0.54 (-0.06, 1.14)
Length Z	0.09 (-0.30, 0.48)	0.07 (-0.35, 0.49)	Fat Free Mass Z	0.08 (-0.31, 0.47)	0.04 (-0.36, 0.44)
Head Circ. Z	0.28 (-0.09, 0.65)	0.40 (0.01, 0.80)	Body Fat % Z	0.75 (0.14, 1.35)	0.58 (-0.05, 1.21)
Total Brain	4.31 (-10.9, 19.6)		15.3 (-1.13, 31.7)		
Cortical Gray Matter	2.60 (-6.89, 12.1)		8.71 (-1.48, 18.9)		
Deep Gray Matter	0.30 (-0.89, 1.49)		0.92 (-0.37, 2.20)		
White Matter	2.34 (-6.34, 11.0)		2.77 (-6.63, 12.2)		

Values are β (95% CI); *Pseudomonas-Staphylococcus* lactotype = reference group. Adjusted for gestational age, sex, birthweight z-score, PMA at assessment.

Summary

Mean ± SD maternal age was 31.9 ± 5.3 years; 50% identified as White. Very preterm infants were born at 28.4 ± 2.0 weeks' gestation and had a birthweight of 1147 ± 358 grams (Table 1).

At both human milk collection timepoints, a total of six lactotypes with distinct microbial compositions were identified (Figure 1).

There were no associations of human milk lactotypes at 2 weeks' chronological age with neonatal outcomes at term-corrected age.

Compared to *Pseudomonas-Staphylococcus* at 36 weeks' PMA:

Staphylococcus-mixed had greater head cir. (β=0.4, 95% CI: 0.01, 0.8) and total brain volume (β=15, 95% CI: -1.1, 32) (Table 2)

Staphylococcus-Streptococcus had greater fat mass (β=0.7, 95% CI: 0.1, 1.3) and body fat % (β=0.8, 95% CI: 0.1, 1.4) (Table 2).

Conclusions

Human milk microbiome evolves over the course of NICU hospitalization, and variation in its composition may influence growth and brain development in very preterm infants

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