



Abstract

Development of the Breastfed Infant Oral Microbiome over the First Two Years of Life in the BLOSOM Cohort[†]

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Abstract: Acquisition and development of the oral microbiome are dynamic processes that occur during early life. However, data regarding longitudinal assembly and determinants of the infant oral microbiome are sparse. This study aimed to characterise temporal development of the infant oral microbiome during the first two years of life. Infant oral samples ($n = 667$ samples, 84 infants) were collected at 2–7 days and 1, 2, 3, 4, 5, 6, 9, 12, and 24 months of age using COPAN E-swabs. Bacterial DNA profiles were analysed using full-length 16S rRNA gene sequencing. At 4 months of age, 76.2% of infants were exclusively breastfed, while breastfeeding rates were 83.3% at 6 months and 65.5% at 12 months. The median breastfeeding duration was 12 months (IQR: 3 months). In this cohort, the oral microbiome was dominated by *Streptococcus mitis*, *Gemella haemolysans*, and *Rothia mucilaginosa*. Bacterial richness decreased significantly from 1 to 2 months, then rose significantly from 12 to 24 months. Shannon diversity increased from 1 week to 1 month and again from 6 to 9 months and 9 to 12 months (all $p \leq 0.04$). Microbiome composition was significantly associated with multiple factors, including pacifier use, intrapartum antibiotic prophylaxis, maternal allergy, pre-pregnancy BMI, siblings, delivery mode, maternal age, pets at home, and birth season (all $p \leq 0.03$). Introduction of solid foods was a significant milestone in oral microbiome development, triggering an increase in bacterial diversity (richness $p = 0.0004$; Shannon diversity $p = 0.0007$), a shift in the abundance of seven species, and a change in beta diversity ($p = 0.001$). These findings underscore how the oral microbiome develops over the first two years of life and highlight the importance of multiple factors, particularly the introduction of solid foods, in shaping the oral microbiome during early life.

Keywords: oral microbiome; temporal; infant diet; breastfeeding; human milk



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