

**TITLE: Salivary Microbiome in Infants Three Years Prior the Clinical Diagnosis of ECC**

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**Objectives** Timely identification of early childhood caries (ECC) risk is crucial for its prevention.

Objective: 1) to determine if the ECC-status is already reflected in the salivary microbial composition of the infants three years prior the clinical diagnosis; 2) to assess a relation between dietary factors and salivary microbiome.

**Methods** This is a part of the “Predicting Caries Risk in Underserved Toddlers in Primary Healthcare Settings” study. Unstimulated saliva was collected from 189 one-year-old orally-healthy children and their primary caregivers (94% mothers). Caries status (dmft, ICDAS) was determined at the age of 1, 2.5 and 4 years. Salivary microbial composition was assessed using 16S rDNA amplicon sequencing. Microbiome profiles were ordinated using Principal Component Analysis (PCA) and differences assessed by Permutational Multivariate Analysis of Variance (PERMANOVA). The profiles were compared using the biomarker discovery tool LEfSe and the difference in relative abundances of the zOTUs were assessed using the Kruskal-Wallis test.

**Results** At the age of 4 years, 70 children (37%) had dental caries (dmft>1). Their salivary profiles at one year of age differed significantly ( $p=0.0017$ ) from the caries-free group (N=119). The difference was due to lower proportions of *Porphyromonas*, *Neisseria*, *Fusobacterium*, *Aggregatibacter* and *Haemophilus* and higher proportions of *Streptococcus*, *Actinomyces*, *Rothia*, *Alloprevotella*, *Prevotella*, *Veillonella*, *Gemella* and *Capnocytophaga* ( $p<0.05$ ) in the caries group. Ethnicity, Medicaid status and recruitment site contributed to the observed differences. Dietary habits (e.g., overnight nursing or bottle usage, frequency of sugary drinks) of the infants had significant effects on their salivary microbiome. Interestingly, dietary habits of the caregivers not only significantly influenced their own salivary microbiome, but also those of their children.

**Conclusions** Ecological shift in salivary microbiome had occurred as early as three years before the clinical diagnosis of ECC. Microbial composition was influenced by dietary habits of both the infant and the caregiver.