Dental Plaque Microbiota Profiles of Caries-free and Caries-active Children

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INTRODUCTION

Microbiota comparisons between healthy and diseased dental tissues have accentuated the importance of cultivating and identifying bacterial species that play a role in the initiation and progression of dental caries. The development of new DNA sequencing technologies has allowed for a more in-depth identification of hundreds of previously uncultivable oral bacterial taxa.

Objectives

The objective of this study was to investigate the microbial diversity of dental plaque using Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS) technology in children with active dental caries in their mixed dentitions, and compare it to caries-free children.

METHODS & MATERIAL

- Ethical approval was obtained from the HSC ethical clearance committee, Kuwait University. Written informed consent were retrieved from the parents of all participants.
- Out of a random sample of 2,173 school girls and boys, 128 children participated in this study. They were divided into two groups: the caries-active group, consisting of 64 children, and the caries-free group, also consisting of 64 children.
- *Inclusion criteria*: healthy 6-9 years-old. Group 1: caries-free (dmft and DMFT=0) and without history of caries, and Group 2: caries-active (dt and DT ≥ 2, and DMFT and dmft ≥ 5).
- *Exclusion criteria*: 1) systemic diseases or drug use that could impact oral microbiota or salivary gland functions; 2) history of salivary gland diseases; 3) antibiotic therapy within 3 months; and 4) children with severe localized or generalized periodontitis.
- ***Clinical Examination and Sample Collection***: Participants were examined in the school's dental clinic, where supragingival plaque samples were collected from the buccal/labial, lingual/palatal, and proximal surfaces of all teeth. Plaque collection was performed using a sterile Gracey 1&2 Currette into 1.5ml tubes filled with 1 ml sterile PBS.

Sample Processing, DNA Extraction & HOMINGS 16S rRNA Gene Sequencing

- All vials were transported to the laboratory for processing in an ice container and were centrifuged at 5000×g for 5 min. The supernatants were discarded, and the pellets were stored at -80°C.
- The variable region V3-V4 was sequenced and analyzed.
- Each raw sequence read was searched for the presence of species level probe sequences using 16S rRNA gene-based probes and ProbeSeq, a BLAST program.

Statistical and Bioinformatics Analysis

Microbial community structure and composition analyses were conducted by processing operational taxonomic units (OTUs). Analysis of Similarity, alpha diversity, and beta diversity were performed. Differences in relative abundances of taxa between the two groups were evaluated using the Mann–Whitney U-test. A significance level of $P<0.05$ was considered statistically significant.

RESULTS

The demographics and clinical findings of the study subjects are presented in the table.

	Caries-active			Caries-free		
	Females	Males	Mean (SD)	Females	Males	Mean (SD)
Sample Size	33	31	__	34	30	__
Age (SD) years	7.80 (0.60)	7.86 (0.66)	7.83 (0.57)	7.83 (0.60)	7.83 (0.56)	7.83 (0.57)
Mean DMFT/dmft (SD)	10.27 (3.07)	10.97 (3.02)	10.61 (3.04)	0	0	0
Mean DMFS/dmfs (SD)	26.36 (13.99)	30.03 (14.82)	28.14 (14.40)	0	0	0
Mean DT and dt (SD)	7.03 (2.24)	8.84 (3.16)	7.91 (2.85)	0	0	0
Mean DS and ds (SD)	12.49 (7.98)	20.03 (13.18)	16.14 (11.38)	0	0	0
Mean DFT and dft (SD)	8.55 (2.17)	9.81 (2.93)	9.16 (2.62)	0	0	0
Mean DFS/dfs (SD)	17.12 (8.19)	22.42 (13.21)	19.69 (11.15)	0	0	0

On average, 79,143 of sequences were generated for each sample (range 38,298-147,401), out of which 30% and 49% were identified at the genus and the species levels, respectively. Diversity indices did not show differences between the two groups ($p>0.05$). Analysis of Similarity demonstrated that the microbiota composition between the two groups did not differ. The principal coordinate analysis did not separate the two groups. Comparative analysis at the species level (fig 1) revealed a significantly higher relative abundance of Leptotrichia shahii, Prevotella melaninogenica, Veillonella dispar, Leptotrichia HOT 498, and Streptococcus mutans in caries-active children ($p<0.05$). Corynebacterium matruchotii, Lautropia mirabilis, Neisseria elongata, and Corynebacterium durum were relatively more abundant in the caries-free group ($p<0.05$). Species belonging to the Leptotrichia, Prevotella, and Veillonella genera were significantly predominant in the caries-active subjects (fig 2).

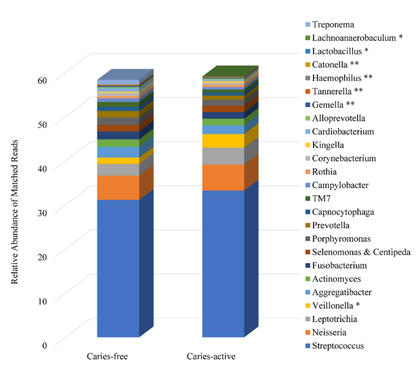

Fig. 1. Relative abundance levels of the top 25 most predominant GENERA (* significantly more in the caries-active and ** significant more in caries-free children)

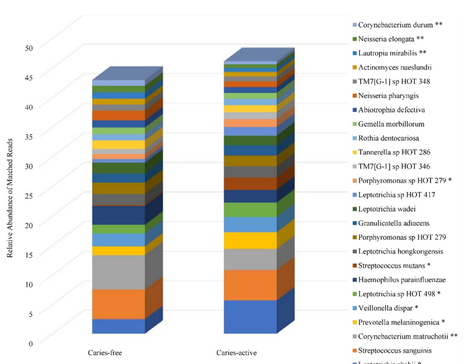

Fig. 2. Relative abundance levels of the top 25 most predominant SPECIES (* significantly more in the caries-active and ** significant more in caries-free children)

CONCLUSION

These findings corroborate several recent reports. Nevertheless, the lack of a clear association of Corynebacterium spp with caries versus health in the literature necessitates further research. The predominance of this species in orally healthy children warrants investigation to better understand its potential role in a health-associated microbial community.

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