Distinct oral bacterial communities observed between tobacco users and non-users: a longitudinal oral microbiome study

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**Background and Objective**

Over 700 bacterial species reside in the human oral cavity, collectively known as the oral microbiome, and these commensal bacteria are involved in different functions, many of which are important in maintaining oral health. Tobacco use is recognized to adversely affect every organ of the human body and influence changes in the oral microbiota. However, less is known about how specific tobacco products cause dysbiosis of the human oral microbiota over time. Here, we aimed to identify the temporal shifts in the bacterial communities in the oral cavity of tobacco users and compare the microbiota profiles from cigarette users and smokeless tobacco users.

**Methods:**

Methods were used to compare the microbiota profiles from cigarette users and smokeless tobacco users.

**Figure 1:** Longitudinal cohort study design

**Results:**

Linear discriminant analysis computed for top 15 differentially abundant bacterial genera between (A) sample type and (B) user groups. The log10 transformed LDA scores are shown on the x-axis and the bacterial taxa are listed on the y-axis. The color coding in the squares of the right side of the plot refers to the cumulative abundance of each genus in each binned group, red means high cumulative abundance and blue means low cumulative abundance. CG: cigarette user; NU: non-user; ST: smokeless tobacco user.

**Figure 2:** Linear discriminant analysis computed for top 15 differentially abundant bacterial genera between (A) sample type and (B) user groups. The log10 transformed LDA scores are shown on the x-axis and the bacterial taxa are listed on the y-axis. The color coding in the squares of the right side of the plot refers to the cumulative abundance of each genus in each binned group, red means high cumulative abundance and blue means low cumulative abundance. CG: cigarette user; NU: non-user; ST: smokeless tobacco user.

**Figure 3:** Average relative abundance (± SE) of top six bacterial genera present in all samples across four time points.

**Figure 4:** Differential relative abundance of bacterial OTUs in (A) buccal mucosa and (B) saliva samples that were statistically significantly different (p<0.05) between time-point 1 and 4. A positive log2-fold change value denotes an OTU that is significantly higher in time-point 1 samples, while a negative log2-fold change indicates an OTU that is significantly higher in time-point 4 samples. The grey line highlight the conversion in log2-fold change from negative to positive values.

**Discussion and conclusion:**

- Oral bacterial communities are dominated by Streptococcus, Prevotella, Haemophilus, Neisseria, and Veillonella, regardless of tobacco use status (non-user, cigarette user, and smokeless user).
- Tobacco users had a larger and more diverse set of core bacterial species, with either significant co-occurrence or co-exclusion relationships, than non-users.
- Tobacco users had a lower relative abundance of Proteobacteria compared to non-users. Haemophilus was significantly higher in smokers when compared to non-users, while a smoker showed lowest impact by smoking status.
- Cigarette users showed a significantly higher relative abundance of Actinobacteria in saliva when compared to non-users. Porphyromonas showed higher abundance in saliva compared to non-users.
- Significant difference in the diversity of bacterial communities was also observed between buccal mucosa and saliva samples. Haemophilus, Lactobacillus, Pseudomonas, and Rothia showed higher while Leptotrichia, Orbacterium, Porphyromonas, and Prevotella showed lower relative abundance in buccal mucosa samples when compared to saliva samples.
- Our results indicate smoking using tobacco products significantly changes the oral microbiome, even though it remains consistent over time. Alterations in the prevalence of some of the opportunistic pathogens in the smokers’ samples indicate poor oral health, which could lead to the initiation of smoking-related diseases.
- Overall, these results provide a more holistic understanding of the structure of microbial communities in the oral cavity, and some clues about how smoking exposures may govern their assembly and lead to disease states.

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