EFFECTS OF ASPIRIN INTERVENTION ON HEALTH AND DISEASE-ASSOCIATED ORAL BACTERIAL TAXA

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Introduction

- Several bacterial taxa which are consistently enriched in the gut microbiome of CRC cases are also found in the oral cavity.
- These bacteria exhibit pathogenic phenotypic traits, such as adherence to host epithelial cells, mucus degradation and biofilm formation
- Previous evidence suggests those traits promote bacterial survival in the colon and may play a role in both oral disease and colorectal carcinogenesis by stimulating an inflammatory response.

Goal of the study

- We evaluated the effect of a 6-week aspirin intervention on the relative abundance of oral bacterial taxa in a randomized double-blinded placebo-controlled trial.
- **Hypothesis:** Intervention assignment was hypothesized to influence the following bacterial taxa a priori: *Gemella, Campylobacter, Porphyromonas, Actinomyces, Prevotella, Fusobacterium, Streptococcus, Neisseria, Veillonella,* and *Haemophilus,* as presented in figure 2.
  - The bacterial taxa were chosen based on previous studies.

Methods

- Fifty healthy subjects, 50-75 years old, were randomized to receive either aspirin (N=30) or placebo (N=20) for 6 weeks.
- Oral samples were collected at baseline (Collection 1) and after 6 week of treatment (Collection 2) .
- Amplicon sequencing of the V4 region of the 16S rRNA gene was done using Illumina MiSeq technology.
- The sequenced data were processed using the standard DADA2 and QIIME2 workflow.
- **Statistical analysis**: We assessed the association between intervention assignment (aspirin vs. placebo) and the following microbiome measures

  •**Alpha Diversity**: linear regression analysis, non-parametric paired sample analysis, and linear mixed effects models.

  •**Beta Diversity**: PERMANOVA (Adonis function) and a non-parametric paired analysis.

  •**Changes in the relative abundance of the specified taxa from pre- to post-treatment (baseline to week 6)** a mixed effect regression model (lme4 package) with a binomial distribution. Log of odds ratio (β estimate) for the interaction term (treatment*time) compared aspirin to placebo intervention for post- versus pre-treatment.

  •**Differential Abundance**: negative binomial regression (DESeq2 package) for fold change in the differential abundance of specific taxa. In addition, we used a newly develop differential ranking algorithm (Songbird) to sort the bacterial taxa relative to other taxa in the placebo vs the aspirin group after 6 week of intervention in an exploratory analysis.

Figure 1. ASMIC Trial diagram.

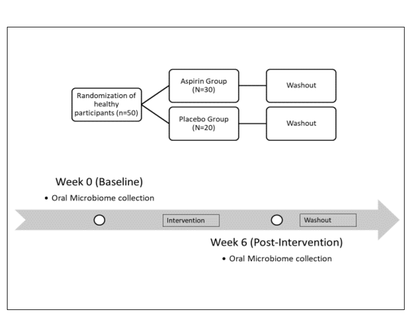
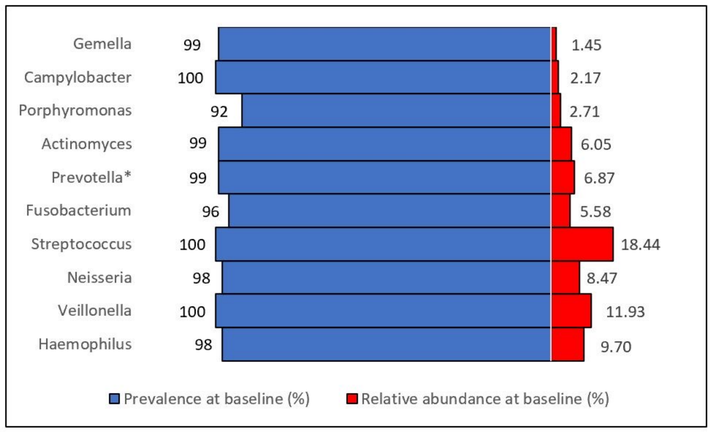
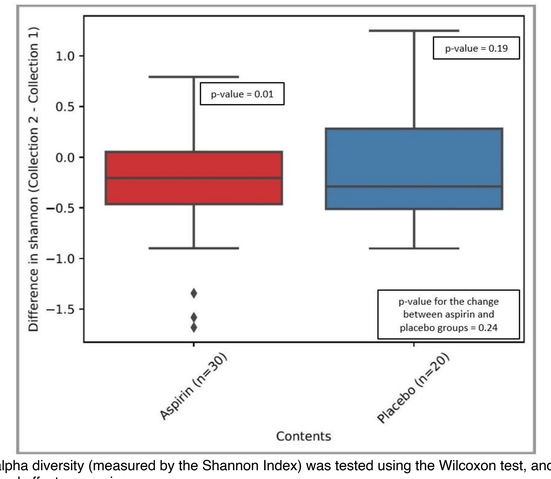


Figure 2. Distribution of pre-specified oral taxa at collection 1.



Prevalence represents the detection prevalence (%).

Figure 3. Change in alpha-diversity between collection 1 and 2.



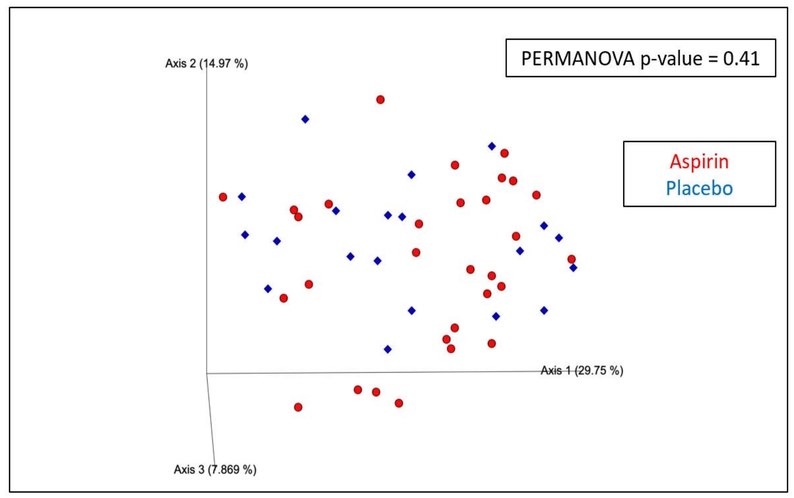
Pairwise changes in alpha diversity (measured by the Shannon Index) was tested using the Wilcoxon test, and overall changes were tested using linear mixed effect regression.

Table 1. Effect of aspirin treatment on the change overtime in abundance of pre-specified bacterial taxa using linear mixed effect models.

Taxa	Predictor	Average Change in relative Abundance (Aspirin)	Average Change in relative Abundance (Placebo)	Estimate	Std. Error	Z Value	Pr(>|z|)
Gemella	Placebo (vs. Aspirin)			0.160	0.204	0.783	0.434
	Collection 2 (vs. Collection 1)	0.014	0.015	0.129	0.084	1.533	0.125
	Intervention * Collection Interaction			-0.052	0.134	-0.385	0.7
Campylobacter	Placebo (vs. Aspirin)			-0.161	0.176	-0.914	0.36083
	Collection 2 (vs. Collection 1)	0.019	0.017	-0.190	0.059	-3.219	0.00129
	Intervention * Collection Interaction			-0.197	0.099	-1.988	0.04678
Porphyromonas	Placebo (vs. Aspirin)			0.156	0.321	0.486	0.6272
	Collection 2 (vs. Collection 1)	-1.252	-0.193	-0.413	0.050	-8.233	<2e-16
	Intervention * Collection Interaction			0.403	0.065	6.230	4.67E-10
Actinomyces	Placebo (vs. Aspirin)			0.131	0.186	0.702	0.483
	Collection 2 (vs. Collection 1)	0.981	-0.554	0.244	0.023	10.729	<2e-16
	Intervention * Collection Interaction			-0.471	0.036	-13.061	<2e-16
Prevotella_7	Placebo (vs. Aspirin)			-0.093	0.181	-0.514	0.60725
	Collection 2 (vs. Collection 1)	-1.191	-1.135	0.050	0.021	2.426	0.01525
	Intervention * Collection Interaction			-0.089	0.032	-2.785	0.00535
Fusobacterium	Placebo (vs. Aspirin)			-0.155	0.300	-0.517	0.605
	Collection 2 (vs. Collection 1)	-1.580	-0.854	-0.203	0.023	-8.969	<2e-16
	Intervention * Collection Interaction			0.160	0.036	4.500	6.80E-06
Streptococcus	Placebo (vs. Aspirin)			0.238	0.202	1.182	0.237
	Collection 2 (vs. Collection 1)	6.498	4.704	0.429	0.009	46.655	<2e-16
	Intervention * Collection Interaction			-0.109	0.013	-8.138	4.01E-16
Neisseria	Placebo (vs. Aspirin)			0.267	0.341	0.783	0.433
	Collection 2 (vs. Collection 1)	2.409	-0.033	0.461	0.016	28.640	<2e-16
	Intervention * Collection Interaction			-0.294	0.024	-12.025	<2e-16
Veillonella	Placebo (vs. Aspirin)			0.016	0.112	0.140	0.8888
	Collection 2 (vs. Collection 1)	-1.653	-0.995	0.003	0.013	0.222	0.8247
	Intervention * Collection Interaction			-0.094	0.020	-4.697	2.64E-06
Haemophilus	Placebo (vs. Aspirin)			-0.033	0.192	-0.174	0.8616
	Collection 2 (vs. Collection 1)	-0.018	-0.556	0.075	0.015	4.989	6.08E-07
	Intervention * Collection Interaction			0.039	0.024	1.638	0.1014

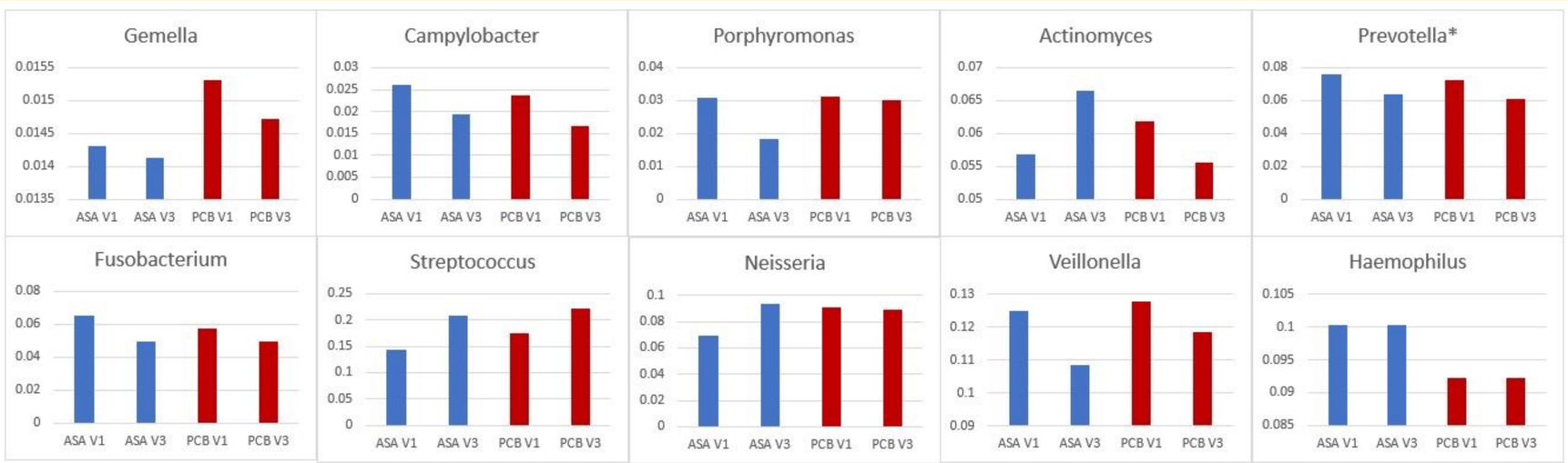
Linear mixed effect regression was used to test for changes in the relative abundance of specific taxa between the aspirin and placebo group after accounting for changes in microbiome composition between collection 1 (Week 0) and collection 2 (week 6)

Figure 4. Change in beta-diversity at collection 2.



Changes in beta-diversity (Bray-Curtis distances) were evaluated at collection 2 between the aspirin and placebo groups using the Adonis function (PERMANOVA)

Figure 5. Change in relative abundance of pre-specified taxa in the aspirin and placebo group from collection 1 (baseline) to collection 2 (week 6).



Changes in relative abundances (%) in the aspirin group (ASA) and placebo group (PCB) between collection 1 (Week 0) and collection 2 (week 6). Pre-specified oral taxa were selected based on their role in CRC and their overall abundance in the oral samples.

Results

- Aspirin treatment was not associated with α- or β- diversity overall after 6 weeks of intervention (collection 2).
- We did not detect differentially abundant taxa on a log fold change scale after 6 weeks of intervention (collection 2) but we found a shift in the balance of taxa which were more prevalent in the placebo group (compared to the aspirin group) (β estimate for the top quintile vs the bottom quintile = 0.85, p = 0.003)
- In the aspirin group, there were greater increases in the relative abundances of *Neisseria, Streptococcus, Actinomyces*, and greater decreases in the relative abundance of *Prevotella, Veillonella, Fusobacterium* and *Porphyromonas*

Conclusions

- These preliminary findings suggest that aspirin may change the relative abundance of oral taxa associated with oral dysbiosis or CRC.
- Further studies are needed to understand the impact that the duration and dosage of the aspirin intervention may have on the oral microbiome.

Acknowledgments

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