

Do genetics, environment, and lifestyle all affect the oral microbiome? A comparative study of the oral microbiome between Amish, Non-Amish, and twins.



Brittany Custer* and Debra Wohl
Elizabethtown College, Elizabethtown, PA

Abstract: Monozygotic twins, also known as identical twins, share great genetic overlap and often share similarities in environment and lifestyle choices. To understand the oral microbiome, we examined monozygotic twins compared with others of similar and differing community, environment, and lifestyle choices. Oral microbiomes are known as communities of microorganisms inside of the oral cavity that can be commensal, symbiotic, or pathogenic. Saliva samples were collected from six Non-Amish females and twelve Amish females, including one pair of monozygotic twins, all between the ages of thirty and sixty years. Community DNA was extracted and the V4 region of the 16S gene was amplified and sequenced. Sequenced DNA was trimmed at 252 base pairs and Phred scores above thirty were obtained indicating high quality sequence data. Based on published literature of the oral microbiome, it was predicted that all of the samples, regardless of population (i.e., Amish and Non-Amish), would have a similar core set of organisms in the oral microbiome. We predict, however, that community composition of the microbiome will differ significantly between the Amish and Non-Amish populations due to differences in genetics, environment, and lifestyles. Because the Amish monozygotic twins share the largest overlap in genetics, environment, and lifestyle, we predict their microbiomes will share the greatest similarities. These predicted results may support our understanding of how environment and genetics affect microbial composition; however, more studies need to be done to fully determine the cause of such results.

Introduction:

- A microbiome is a community of microorganisms within the body that can be commensal, symbiotic, or pathogenic.

- Structured, regulated, organized communities which can adhere to surfaces
- Determined by factors such as age, gender, genotypes, and health status
- More closely related individuals share more similar microbiomes
- Twins were found to have higher similarities in their gut microbiomes than their parents or other siblings

- The microorganisms in the oral cavity are in a symbiosis while also helping to keep pathogenic microbes from adhering to mucosal surfaces.
- Some studies have found differences in certain oral microbiomes based on ethnic differences.

- Amish populations are unique in their lifestyle choices, specifically their dietary and hygiene habits.

Results:

-The five most abundant phyla in both populations were Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes, and Fusobacteria.

Hypothesis: Community composition of the oral microbiome will differ significantly between the Amish and Non-Amish populations. Compared to other study participants, the Amish monozygotic twins will share the largest similarity in their microbiomes.

Table 1: Pairwise Comparison of Species Richness

Pairs	p-value
Twins: Non-Amish	0.207
Twins: Amish	1
Amish: Non-Amish	0.042

Table 2: Mean number of species in the samples \pm standard deviations

Group	Richness Mean \pm Std
Twins	119.425 \pm 0.425
Amish	111.475 \pm 18.713
Non-Amish	73.175 \pm 31.877

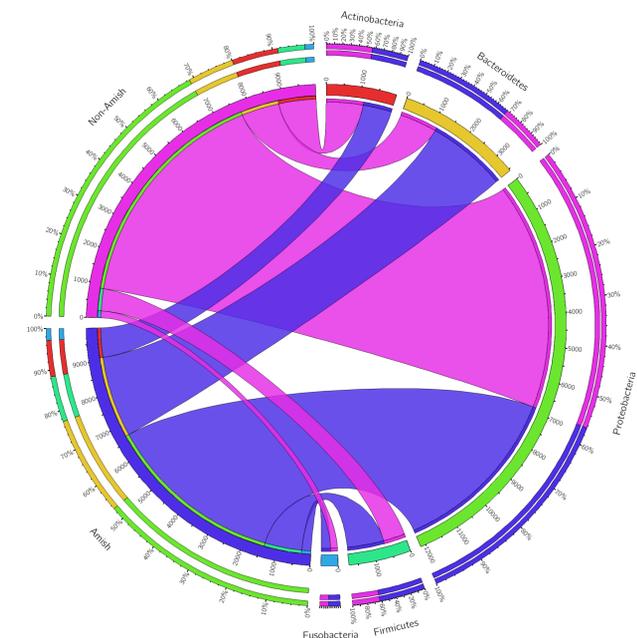


Figure 2: Five most abundant phyla and their weighted distribution between groups

Materials and Methods:

- Saliva samples were collected from 12 Amish and 6 Non-Amish women between the ages of 30 and 60 years at their dentist's offices
- DNA was extracted from samples using MoBio Bacterimia Kit
- PCR was used to amplify the V4 region of the 16S rRNA gene
- Sequenced using the Illumina MiSeq Platform

- Sequence data was merged and filtered
- Metadata labels were added before OTUs were assigned
- Taxonomy was determined

-Using Qiime, non-parametric tests were used to compare significance between samples

- Venny produced the Venn diagram and Circos produced weighted taxa distribution

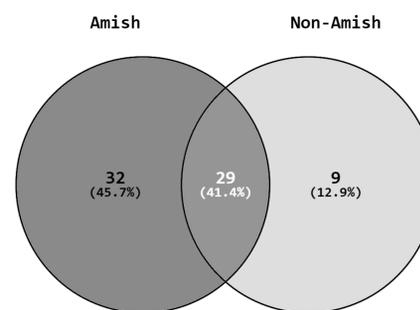


Figure 1: Core OTUs present within 95% of the samples

Discussion:

- No significant difference in richness was found between the Twins and the Amish ($p=1.00$). Core OTUs for Twins and Amish overlapped (data not shown). Given that these groups also have similar community, environment, and lifestyle choices, they were grouped together for further analyses.
- The Amish and Non-Amish have a significantly different number of OTUs in their respective core saliva microbiomes (Amish=61, Non-Amish= 38)
- The Proteobacteria and Actinobacteria were similarly represented between both groups. The Bacteroidetes and Firmicutes were more abundant in the Amish samples.
- More analysis needs done to determine how the twins' oral microbiome composition differs from the Non-Amish oral microbiome composition.

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