



# Classification of the Oral Microbiome: Comparing the Amish and Non-Amish Communities

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## Abstract:

The oral cavity consists of several habitats suitable for bacterial colonization comprised of the hard and soft palates, along with the supragingiva, tongue and teeth. The sum of interactions between microorganisms and their environment constitute a microbiome. The oral microbiome offers niches specific for innocuous bacteria to reside in while resisting the invasion of bacteria that causes dental carries or periodontal disease. The aim of this study is to elucidate the similarities and dissimilarities of the oral microbiome between the Old Order Amish and the non-Amish population of the central Mid-Atlantic States. We hypothesized that the oral microbiomes of the two communities would be distinct due to varying dental and dietary practices. Supragingival plaque samples were obtained from volunteers along with oral health questionnaires; community DNA was extracted, the V4 region of the 16s rRNA gene was amplified using Polymerase Chain Reaction (PCR) and further analyzed using Illumina and QIIME. The study enrolled 46 participants. Selection criteria limited the study to individuals between the age of 20 and 70 with no indication of periodontal disease, which reduced the sample size to 29 participants: 12 non-Amish and 17 Amish. Based on our oral health questionnaire, the non-Amish are more likely to prioritize oral hygiene (i.e., brushing, flossing, and visiting a hygienist regularly) in comparison to their Amish counterparts. Further analyses will identify any variances between the oral microbiome of the two communities. We anticipate the Amish and non-Amish supragingival plaque samples will reflect differences in the community composition, such as, *Streptococcus mutans* associated with dental carries. Oral microbiome data along with health questionnaires may help elucidate how the composition of the oral microbiome resists the colonization of the bacteria that cause oral health problems.

## Introduction:

- The human oral cavity consists of numerous habitats such as the soft and hard palates, the supragingiva, tongue, and teeth.
- The microorganisms that colonize an environment, such as the oral cavity, constitute a microbiome.
- Many innocuous bacteria are present in the oral microbiome and prevent colonization of pathogenic bacteria by competing for resources.
- While the core microbiome has a degree of stability, species abundance and richness can vary with diet and oral health practices.
- Literature has reported that due to the high degree of isolation, Amish communities exhibit differences in diet and dental practices in comparison to the Non-Amish community.
- By elucidating dissimilarities between the oral microbiomes of Amish and Non-Amish we may gain a more comprehensive understanding of the dynamics acting within the oral microbiome.

## Hypothesis:

The oral microbiome differs between the Amish and Non-Amish communities due to dental and dietary practices.

## Objectives:

1. To identify and analyze microbial diversity within the oral cavity
2. To elucidate differences in plaque composition between Non-Amish and Amish individuals aged 20 to 70 years.

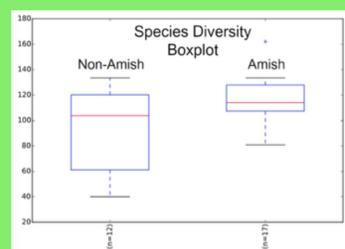
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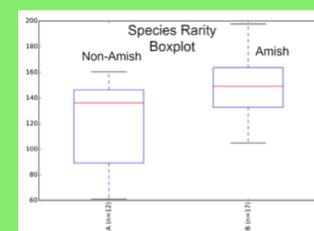
## Results:

**Table 1.** The tabulated percentages gathered from the Oral Health Questionnaire on the frequency of dental visits for the Amish and Non-Amish (n=18, 27 respectively).

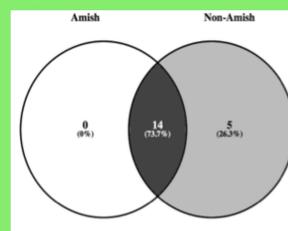
Duration Between Dentist Visits	Amish	Non-Amish
Every 6 months or more	0%	55.6%
Every 6 months- 1 year	16.7%	33.3%
Every 5 years	38.9%	0%
Less than Every 5 Years	33.3%	11.1%



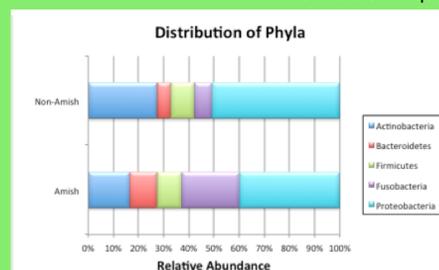
**Figure 1.** Box plot depicts Species Diversity distribution between the Amish and Non-Amish. Non-Amish richness was significantly different than the Amish with a p-value of 0.018.



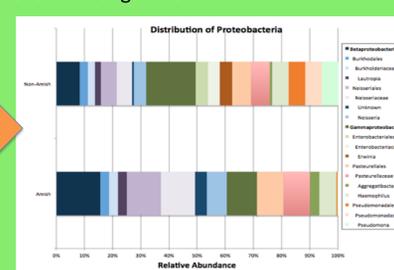
**Figure 2.** Boxplot Depicts Species Rarity distribution between the Amish and Non-Amish. Non-Amish rare taxa were significantly different than the Amish with a p-value of 0.009.



**Figure 3.** Venn diagram depicts percent differences and similarities of Proteobacteria OTUs between the Amish and Non-Amish communities. The Non-Amish had 5 unique OTUs not present amongst the Amish.



**Figure 4.** Depicts the relative abundance of phyla found within the oral cavity from both communities.



**Figure 5.** Depicts the relative abundance of Proteobacteria subtypes.

## Materials and Methods:

- Individuals were invited to the study and asked to complete an oral health questionnaires (n=45)
- Supragingival plaque samples were collected from 18 Amish individuals and 27 non-Amish individuals.
- Individuals between the ages of 20-70 years and no history of periodontal disease were retained for the study
- DNA from plaque samples was extracted using MoBio Bacterimia kit
- The 16s rRNA gene was amplified using PCR
- Data were sequenced using Illumina
- Sequence Analyses:**
- Sequences were trimmed at 252 base pairs
- Using QIIME, non-parametric tests were used to compare two-sample T-test to determine significance

## Discussion:

- Based on selection criteria, data from 17 Amish and 12- Non Amish individuals were analyzed
- Oral practices differed between communities (Table 1).
- The microbiome of the Amish and Non-Amish differed significantly in species richness: p-value= 0.018 and species rarity: p-value=0.009 (Figs 2 & 3).
- Overall, the Proteobacteria phylum composed the greatest proportion of bacteria in both sample populations (Fig. 4).
- Of the 19 Proteobacteria OTUs identified, only 5 were unique to the Non-Amish community (Fig.1).
- Beta-proteobacteria and Gamma-proteobacteria were prominent in both communities (Fig. 5).

**There are significant differences in the oral microbiome between Amish and Non-Amish which can be attributed to dental practices. Despite these differences, there is a common core group of microorganisms.**

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