H109 An Investigation Into the Relationship of Postmortem Interval and Bacterial Metagenomics of Bone

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After attending this presentation, attendees will gain an understanding of the relationship between Postmortem Interval (PMI) and bacterial metagenomic profiles recovered from human skeletal remains.

This presentation will impact the forensic science community by providing baseline data for exploring the relationships among bacteria, cadaver decomposition, and estimating PMI.

Microbially mediated decomposition of a corpse leaves signatures in bone and their deleterious effects on structural integrity of bone have been defined previously.[1] The amount of bone destruction due to microbial activity has been loosely correlated with PMI, which has been observed occurring later than five years after death.[2] Yet bacteria are involved in postmortem processes immediately following death.[3] Thus, the goal of this research is to produce a community survey of bacterial phyla present in bone of varying PMIs.

Previous analyses of microbial concentration in bone from the University of Tennessee Anthropology Research Facility (UTARF) demonstrated an inverse relationship between total bacterial load and PMI.[4] This study used the same skeletal tissue to determine the bacterial composition of those samples. In doing so, the relative contribution of specific bacterial phyla was expected to change with advancing decomposition, as the sampled bodies transitioned from a high-quality to a low-quality resource.

Eleven human ribs sampled from as many actively decomposing corpses were analyzed. The corpses spanned a PMI of 1 – 48 months. Three soil samples with no known history of human decomposition were also analyzed in order to compare bacterial communities between decomposing bone and "normal" soil. Total DNA was extracted and next-generation sequencing was used to amplify and sequence a 200-base-pair product of the universal eubacterial 16S rRNA gene.[5,6] Recovered sequences were trimmed, aligned, and classified using the Ribosomal Database Project (RDP) pipeline and classifier.[7,8] Identified phyla were analyzed using cluster analysis on standardized abundance data.

Sequences from all rib samples provided 124,164 classified sequences. Results indicated consistency in the presence of specific bacterial phyla. The six most abundant phyla across all bone samples accounted for 94.37% of all classified bacterial phyla. The six most abundant phyla were: Protebacteria (64%), Firmicutes (12%), Bacteroidetes (10%), Actinobacteria (7%), Acidobacteria (1%), and Gemmatimonadetes (0.26%). Nearly 6% (5.62%; n=6,976) of the trimmed and aligned sequences remained unclassified at the bacterial phylum level. Chloroflexi, TM7, and Deferribacteres were consolidated into a single group "other" due to low representation (0.008%). Cluster analysis and MDS mapping on bacterial abundance data identified three significantly different clusters ($p<0.05$). One cluster was characterized by samples 1.2, 2, and 12.3 months. The second group consisted of samples with PMI of 7 – 20 months. The third cluster was composed of the non-UTARF soil samples and the 24- and 48-month PMI bone samples.

In all samples, Proteobacteria were the most abundant and were therefore determined to be of little value as a marker for evaluating temporal trends in phylum-level bacterial community structure. The greatest change in relative abundance occurred among the Firmicutes, Bacteroidetes, and Actinobacteria. The early PMI samples were dominated by Firmicutes. Bacteroidetes dominated the second phase of samples from 9 to 20 months, with the exception of one 12-month sample. The 24- and 48- month samples were dominated by Actinobacteria, and the level of Acidobacteria increased between these two samples, which was consistent with the non-UTARF soil samples.

These results provide baseline data for further exploration regarding the relationships among bacteria, cadaver decomposition, and PMI. Within these data, a succession in the relative abundance of three bacterial phyla was observed, demonstrating the potential for using bacterial metagenomics as a method for estimating PMI of decomposed skeletal remains.

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Taphonomy, Postmortem Interval, Bacterial Metagenome

